IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	: Thomas Julius BORODY	Art Unit	: 1616
Serial No.	: 10/506,728	Examiner	: Holt, Andriae M.
Filed	: June 27, 2005	Confirm. No.	: 7029

Title : **ELECTROLYTE PURGATIVE**

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Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

PETITION PURSUANT TO 37 C.F.R. §1.181 FOR RECONSIDERATION OF THE FINDING OF LACK OF UNITY AND WITHDRAWAL OF THE RESTRICTION REQUIREMENT

Dear Sir:

Applicant hereby submits a Petition pursuant to 37 C.F.R. §1.181 for reconsideration of the finding of lack of unity and withdrawal of the requirement for restriction made final in the Office Action, mailed July 7, 2008, in connection with the above-captioned application.

Remarks begin on page 2 of this paper.

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I hereby certify that this paper is being deposited with the United States Postal "Express Mail Post Office to Addressee" Service under 37 CFR §1.10 on the date indicated above and is addressed to: Mail Stop Petition, Commissioner for Patents, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA, 22313-1450.

Kethya Teuk

REMARKS

The fee for filing this Petition and any other fees that may be due in connection with the filing of this paper or with this application may be charged to Deposit Account No. 02-1818. If a Petition for Extension of Time is needed, this paper is to be considered such Petition. This Petition is filed within two months of the mailing date of the Office Action in which the requirement for restriction was made final, and therefore is filed within the period recited in 37 CFR 1.181(f).

Applicant respectfully submits that there is a novel common underlying technical feature shared among all pending claims. Therefore, all pending claims (*i.e.*, Groups I-V) are unified and should be examined in this application.

1. The Restriction Requirement

The Office Action, mailed November 30, 2008, restricted the pending claims into ten groups as follows:

Group I:	claims 1-9, drawn to a composition for use in a purgative;
Group II:	claims 10-11, drawn to a method of inducing purgation of the colon;
Group III:	claims 12-13, 18 and 36, drawn to methods of treatment or prevention of one or more of the listed conditions;
Group IV:	claims 14-15, drawn to a method for the treatment or prevention of acute gastrointestinal infections;
Group V:	claims 16-17, drawn to a method for the treatment or prevention of constipation;
Group VI:	claims 19-21, drawn to a composition for the use in a purgative;
Group VII:	claims 22-23, drawn to a method of inducing purgation of the colon;
Group VIII:	claims 24-25 and 30-35, drawn to a method for the treatment or prevention of one or more of the listed conditions in the absence of diathermy;
Group IX:	claims 26-27, drawn to a method for the treatment or prevention of acute gastrointestinal infections; and
Group X:	claims 28-29, drawn to a method for the treatment or prevention of constipation.

The Examiner, applying the rules of unity of invention under PCT Rule 13.1, alleges that a lack of unity exists because the ten groups allegedly do not relate to a single inventive concept. This conclusion is based upon the premise that a special technical feature shared among the claims is disclosed in Kang *et al.* (US Pat. 4,186,025, issued January 29, 1980). The Examiner alleges that the common technical feature among the claims is a composition that includes (i) at least one water soluble sodium salt; (ii) at least one water soluble minimally degradable sugar; (iii) at least one water soluble potassium salt; and (iv) at least one water soluble magnesium salt. The Office Action alleges that the claims of Groups I - V lack unity

of invention because the composition of claim 1 allegedly is not novel. The Examiner alleges that Kang *et al.* (U.S. Pat. No. 4,186,025) discloses a fermentation medium that includes a hydrolyzed starch, a source of magnesium ions, a source of phosphorous, a source of nitrogen and water, and that this composition destroys novelty of the compositions of claim 1 and unity of invention among groups I-V.

Claims 1-18 and 34-39 are pending. Applicant petitions the propriety of the restriction as between and among Groups I-V.

2. The Pending Claims

Claim 1 of Group 1 recites:

A composition for use in a purgative, the composition comprising:

(i) at least one water-soluble sodium salt;

(ii) at least one water-soluble minimally degradable sugar in an amount, by weight, of from about 1 to about 3 times the weight of sodium salt in the composition;

(iii) at least one water-soluble potassium salt in an amount, by weight, of from about 0.05 to about 1 times the weight of the sodium salt in the composition; and

(iv) at least one water-soluble magnesium salt, wherein the weight of magnesium salt in the composition is from about 0.1 to about 10 times the weight of sodium salt in the composition.

The claims of Group II are directed to methods of inducing purgation of the colon by administering a composition of claims of Group I.

The claims of Group III are directed to methods of treatment or prevention of lavage-associated hyponatremia, hypoosmolality, nausea, malaise, vomiting, headache, convulsions by administering a composition of claims of Group I and methods of pre-colonoscopic or pre-surgical lavage using a composition of claims of Group I.

The claims of Group IV are directed to methods for the treatment or prevention of acute gastrointestinal infections by administering a composition of claims of Group I.

The claims of Group V are directed to methods for the treatment or prevention of constipation by administering a composition of claims of Group I.

Hence, the composition of claim 1 is a technical feature shared among all pending claims. As discussed below, the composition of claim 1 is novel over Kang *et al.* Thus, the composition of Group I (*i.e.*, claim 1) is novel and is a common technical feature in all of the pending claims. Therefore, all pending claims are unified.

3. Finality of requirement for restriction

The Office Action, mailed July 7, 2008, states that Applicant's election, with traverse, was timely filed and considered. In response to the traverse, the Examiner rejoined the method claims of Group II with the composition claims of Group I, but maintains the requirement as

between and among Group I and Groups III-V. The Examiner alleges that Kang *et al.* discloses every element of claim 1 because it discloses a fermentation medium that:

> contains a carbon source, preferably a hydrolyzed starch, a source of magnesium ions, a source of phosphorous, a source of a nitrogen and water. Kang *et al.* teach suitable sources of magnesium ions include water soluble magnesium salts. Kang *et al.* teach that at least a trace quantity of phosphorous generally in the form of a soluble potassium salt is present in the fermentation medium. Kang *et al.* further teach that if the pH drops sodium hydroxide may be added to maintain the pH at least about 6.6. These elements cannot be a special technical feature under PCT Rule 13.2 because the elements are shown in the prior art.

4. Disclosure of the Cited Art and differences from the pending claims

Kang *et al.* discloses several fermentation media. None contain a minimally degradable sugar, a sodium salt, a potassium salt and a magnesium salt. Further, as shown below, none of the fermentation media include the requisite weight ratios of components.

A. E-1 medium

Kang *et al.* discloses a fermentation medium (E-1 medium) that contains 0.5% potassium acid phosphate, 0.01% magnesium sulfate, ammonium nitrate, Promosoy and 3% hydrolyzed starch (see col. 2, lines 42-48). The hydrolyzed starch described in Kang *et al.* is not a minimally degradable sugar. Kang *et al.* teaches that its "hydrolyzed starch" refers to the product obtained by hydrolyzing a starch preparation using an α-amylase (*e.g.*, see col. 2, lines 48-58 and col. 9, line 53 through col. 10, line 32), and states that a preferred hydrolyzed starch product has a dextrose equivalent of about 20-30 (see col. 10, lines 9-15). It is known in the art that treating starch with α-amylase results in products that are easily digested. For example, Rooney *et al.* (J Anim Sci 63: 1607-1623 (1986)) at page 1612 recites

> [t]he linear and branched dextrins produced by α-amylases and debranching enzymes are hydrolyzed much faster by intestinal amyloglucosidases than are larger starch molecules.

and Record *et al.* (WO1996002148) states at page 3, line 38 through page 4, line 6:

> Typical maltodextrins are made by acid and/or enzyme hydrolysis of starches. They are hydrolyzed to a Dextrose Equivalent (D.E.) of about 4-27 and are typically spray dried to a powder. They are also readily digestible since the glucose polymer consists essentially of α-1,4 bonds between glucose molecules.

The instant specification defines a "minimally degradable sugar" as a carbohydrate moiety that is substantially resistant to endogenous digestion in the gastrointestinal tract (see page 7, lines 28-30). Hence, hydrolyzed starch as disclosed in Kang *et al.* is not a minimally degradable sugar as defined in the specification because it is readily digestible. Thus, the E-1 medium does not contain a minimally degradable sugar. In addition, the E-1 medium does not include a sodium salt. Because the E-1 medium does not contain a sodium salt, the amount of potassium salt and magnesium salt in the E-1 medium cannot be based on the weight of sodium salt in the

composition. Thus, Kang *et al.* does not disclose every element of the composition of claim 1 as instantly claimed and therefore does not anticipate the composition of claim 1.

B. Nutrient broth media

Kang *et al.* also discloses a nutrient broth medium that contains 0.3% by weight of beef extract and 0.5% by weight of peptone in distilled water (col. 3, lines 29-32). Kang *et al.* discloses including 5% or 7% by weight sodium chloride in the nutrient broth medium (col. 4, lines 57-63). This modified nutrient broth containing sodium chloride does not contain a minimally degradable sugar, a potassium salt or a magnesium salt. Thus, Kang *et al.* does not disclose every element of the composition of claim 1 as instantly claimed and therefore does not anticipate the composition of claim 1.

C. Basal medium

Kang *et al.* also describes a "basal medium" for growing bacteria in order to test for acid production. The basal medium contains 0.5% by weight of a carbon source selected from among arabinose, cellobiose, fructose, galactose, glucose, inositol, lactose, maltose, mannitol, mannose, raffinose, rhamnose, ribose, salicin, sucrose, trehalose, xylose, dextrin, adonitol, dulcitol, inulin, sodium alginate and starch (col. 5, lines 1-40). Kang *et al.* does not disclose selecting a minimally degradable sugar from among its carbon sources. Kang *et al.* does not disclose the other ingredients in its basal medium. Kang *et al.* describes its "basal medium" as "the basal medium described by D.W. Dye, New Zealand Journal of Science, Vol. 5, pages 393-416 (1962)" (col. 5, lines 1-4). Dye describes several growth media for bacteria. Dye describes a medium for growing bacteria to test for the production of acid using various carbon sources. This medium of Dye includes 0.05% $NH_4H_2PO_4$, 0.05% K_2HPO_4, 0.02% $MgSO_4 \cdot 7H_2O$, 0.5% NaCl, 0.1% yeast extract, 1.2% agar and 0.5% of a carbon source. When the weights of these components are expressed based on the weight of the sodium salt in the medium, the carbon source is 1× the weight of the sodium salt, the potassium salt is 0.1× the weight of the sodium salt and the magnesium salt is 0.04× the weight of the sodium salt. Instant claim 1 recites that the weight of the water soluble magnesium salt in the composition is from about 0.1 to about 10 times the weight of the sodium salt in the composition. Thus, because the medium described in Dye and referred to in Kang *et al.* as the "basal medium" includes an amount of magnesium salt only 0.04× the weight of the sodium salt, the basal medium contains about 2.5 times less magnesium salt than the minimum amount of magnesium salt recited in the composition of instant claim 1. Thus, Kang *et al.* does not disclose every element of the composition of claim 1 as instantly claimed and therefore does not anticipate the composition of claim 1.

D. The medium described at columns 8-9

Kang *et al.* also describes a medium at columns 8-9 that includes an oligosaccharide containing from about 3 to about 10 monomer units, such as a hydrolyzed starch, at a concentration of about 1 to about 5% by weight; about 0.005 to about 0.02% by weight of a magnesium salt; about 0.4 to about 0.6% by weight of a sodium or potassium salt of phosphoric acid, such as KH_2PO_4, K_2HPO_4, K_3PO_4, NaH_2PO_4, Na_2HPO_4 or Na_3PO_4 and an organic or inorganic nitrogen source (col. 8, lines 35-41). As discussed above, the hydrolyzed starch of Kang *et al.* is readily digested (see Rooney *et al.* (J Anim Sci 63: 1607-1623 (1986)) at page 1612 and WO1996002148 at page 3, line 38 through page 4, line 6). The instant specification defines a "minimally degradable sugar" as a carbohydrate moiety that is substantially resistant to endogenous digestion in the gastrointestinal tract (see page 7, lines 28-30). Thus, the hydrolyzed starch of Kang *et al.* is not a minimally degradable sugar as defined in the specification. Hence, this medium does not include a minimally degradable sugar.

In addition, the amounts of the ingredients in the medium described at cols. 8-9 of Kang *et al.*, if calculated based on the amount of sodium salt, are not within the scope of the instant claims. Kang *et al.* describes two possible sources of sodium salt in the fermentation medium described at cols. 8-9: a sodium phosphate salt and sodium hydroxide. Kang *et al.* describes controlling the pH of the medium by using a phosphate salt or, conversely, using a pH meter and adding an alkali metal hydroxide to control pH. If the sodium phosphate is taken to be the sodium salt, the amount of sodium salt is 0.4-0.5%. The amount of hydrolyzed starch in the medium is 5%. Hence, the amount of hydrolyzed starch in the medium of Kang *et al.* is about 10 to 13 times the weight of the sodium salt, not about 1 to 3 times the weight of sodium salt as instantly claimed. Therefore, the media at cols. 8-9 of Kang *et al.* does not disclose the element "at least one water-soluble minimally degradable sugar in an amount, by weight, of from about 1 to about 3 times the weight of sodium salt in the composition" as instantly claimed.

In the fermentation medium of Kang *et al.* at cols. 8-9, the amount of magnesium salt is 0.005%-0.02%. Assuming that the sodium salt is sodium phosphate in an amount of 0.4-0.5%, the amount of magnesium salt is about 0.013-0.05 times the amount of sodium salt, not 0.1-10 times the amount of sodium salt as instantly claimed. Hence, the amount of magnesium salt in the medium described at cols. 8-9 of Kang *et al.* is at most half the minimum amount of magnesium salt recited in the instant claims (0.05 times the amount of sodium salt compared to the instantly claimed 0.1-10 times the amount of sodium salt).

If the phosphate salt is taken to be sodium phosphate, then the phosphate salt cannot be the potassium salt of the medium, because Kang *et al.* describes these in the alternative (see col. 8, lines 39-41). The only other source of potassium in this medium is potassium hydroxide, which Kang *et al.* discloses is added in "small quantities" to control pH (see col. 8, lines 48-53). It is not possible to determine the weight of the potassium salt in the medium based on the disclosure of Kang *et al.* in cols. 8-9. Thus, Kang *et al.* does not disclose a composition containing at least one water-soluble potassium salt in an amount, by weight, of from about 0.05 to about 1 times the weight of the sodium salt in the composition.

Thus, the medium described at cols. 8-9 of Kang *et al.* does not contain a minimally degradable sugar, does not recite at least one water-soluble potassium salt in an amount, by weight, of from about 0.05 to about 1 times the weight of the sodium salt in the composition and the amount of magnesium salt in the medium described at cols. 8-9 of Kang *et al.* is at most half the minimum amount of magnesium salt recited in the instant claims. Thus, Kang *et al.* does not disclose every element of the composition of claim 1 as instantly claimed and therefore does not anticipate the composition of claim 1.

5. Analysis

In setting forth and maintaining the lack of unity rejection, the Examiner did not consider the limitations of claim 1 that recite that the sugar is a minimally degradable sugar and that the amounts of minimally degradable sugar, potassium salt and magnesium salt in the composition are based on the weight of the sodium salt in the composition. Specifically, claim 1 recites that the composition includes at least one water-soluble sodium salt; at least one water-soluble minimally degradable sugar in an amount, by weight, of from about 1 to about 3 times the weight of sodium salt in the composition; at least one water-soluble potassium salt in an amount, by weight, of from about 0.05 to about 1 times the weight of the sodium salt in the composition; and at least one water-soluble magnesium salt, wherein the weight of magnesium salt in the composition is from about 0.1 to about 10 times the weight of sodium salt in the composition. Because these elements define the composition, they must be considered. In order for Kang *et al.* to destroy unity among Groups I-V, Kang *et al.* must disclose a composition that includes all of these elements.

Kang *et al.* does not disclose any composition that includes at least one water-soluble sodium salt; at least one water-soluble minimally degradable sugar in an amount, by weight, of from about 1 to about 3 times the weight of sodium salt in the composition; at least one water-soluble potassium salt in an amount, by weight, of from about 0.05 to about 1 times the weight

of the sodium salt in the composition; and at least one water-soluble magnesium salt, where the weight of magnesium salt in the composition is from about 0.1 to about 10 times the weight of sodium salt in the composition. Kang *et al.* discloses a number of fermentation media, none of which includes every element of the composition as instantly claimed. As discussed above, the E-1 medium of Kang *et al.* does not contain a minimally degradable sugar or a sodium salt. The nutrient broth of Kang *et al.* does not contain a minimally degradable sugar, a potassium salt or a magnesium salt. The basal medium of Kang *et al.* contains about 2.5 times less magnesium salt than the minimum amount of magnesium salt recited in the composition of instant claim 1. Finally, the medium described at cols. 8-9 of Kang *et al.* does not contain a minimally degradable sugar and the amount of magnesium salt based on the sodium salt is at most half the minimum amount of magnesium salt recited in the instant claims.

Thus, for at least these reasons, none of the media described by Kang *et al.*, including the E-1 medium, modified nutrient broth, the basal medium or the medium described at cols. 8-9 of Kang *et al.*, includes every element of the composition of claim 1. Hence, Kang *et al.* does not destroy novelty of the composition of claim 1. The composition of claim 1 is a technical feature shared among all claims of Groups I-V. Thus, Groups I-V (claims 1-18 and 34-36) possess unity. Therefore, the claims of Groups III-V should be rejoined with the claims of Groups I and II and examined in this application.

* * *

In light of the above remarks, reconsideration and withdrawal of finding of lack of unity among Groups I-V and the requirement for restriction respectfully are requested.

Respectfully submitted,

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ATTACHMENT

1. Rooney *et al.*, J Anim Sci 63: 1607-1623 (1986).
2. Record *et al.*, WO1996002148 (1996).
3. Dye, New Zealand Journal of Science 5: 393-416 (1962).

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Factors Affecting Starch Digestibility with Special Emphasis on Sorghum and Corn

L. W. Rooney and R. L. Pflugfelder

J Anim Sci 1986. 63:1607-1623.

FACTORS AFFECTING STARCH DIGESTIBILITY WITH SPECIAL EMPHASIS ON SORGHUM AND CORN[1]

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ABSTRACT

Starch exists inside the endosperm of cereals enmeshed in a protein matrix, which is particularly strong in sorghum and corn. Starch digestibility is affected by the plant species, the extent of starch-protein interaction, the physical form of the granule, inhibitors such as tannins, and the type of starch. Among the cereals, sorghum generally has the lowest starch digestibility. The resistance to digestive action of the hard peripheral endosperm layer is largely responsible for this effect. Processing methods such as steam-flaking and reconstitution are effective in raising sorghum digestibility to near that of corn. Waxy sorghum shows consistently higher feeding value than normal sorghum. Both the starch granules and the protein matrix around them are more digestible in waxy grain. The development of new heterowaxy or waxy sorghum hybrids may further increase sorghum feed efficiency.

(Key Words: Feeds, Starch Digestion, Sorghum, Maize.)

Introduction

Starch is the storage polysaccharide of higher plants and a major source for animals. Starch represents about 70 to 80% of most cereal grains, a large percentage of many roots and tubers, and is a major component of many grain legumes, i.e., peas, beans and lentils. The livestock industry depends heavily upon corn, sorghum and barley as major sources of energy and protein. The structure and composition of cereal starches and their interactions with proteins play a major role in the digestibility and feeding value of grain for livestock. The goals of this presentation are to review the major factors that affect the digestibility of starch in cereals. The brief review of starch chemistry offered here emphasizes the effect of starch-protein interactions on the utilization of corn and sorghum by livestock. Other critical factors affecting digestion of corn (Zea mays) and sorghum (Sorghum bicolor) will be reviewed. Comprehensive coverage of starch chemistry and technology can be found in Whistler et al. (1984). Recent reviews on starch digestibility and factors affecting it in humans (Thorne et al., 1983), poultry (Moran, 1982) and foods (Dreher et al., 1984) have summarized most available information. Important earlier work in starch chemistry is described by Kerr (1950), Whistler and Paschall (1965, 1967) and Banks and Greenwood (1975). Whistler (1964) is still an excellent reference for methods of starch analysis.

Chemical Properties of Starch

Starch is a glucan composed of two major types of molecules: amylose and amylopectin (table 1). A minor component, termed "branched amylose," may also be present. Amylose is a linear polymer of α-1,4-linked D-glucose units. The proportion of amylose in starch ranges from 0 to 80%, depending upon the species and the genetic variations within a species. Normal cereal starches contain 20 to 30% amylose, while waxy starches contain little or no amylose. Amylose exists as a helix which forms a blue or purple clathrate with iodine.

Amylopectin is a much larger, branched polymer which is the most abundant component of normal starches. Linear chains of α-1,4-linked D-glucose have α-1,6 branch points every 20 to 25 glucose residues. The resulting structure, although likened to a randomly branched tree, is actually highly organized (French, 1984;

[1] Presented by the senior author at a symposium entitled "Starch Utilization by Ruminants" at the 77th Annu. Meet. of Amer. Soc. of Anim. Sci., August 15, 1985, Univ. of Georgia, Athens.

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TABLE 1. PROPERTIES OF STARCH COMPONENTS

Property	Component	
	Amylose	Amylopectin
General structure	Linear	Branched
Branch points	None[a]	1 per 20 to 25 glucose units
Degree of polymerization (DP)[b]	∿1,000	∿10,000–100,000
Iodine complex color	Intense blue	Reddish-brown
Solution stability	Low	High

[a]Branched amylose may have 1 or 2 α-1,6 branches per molecule.

[b]Number of glucose residues per molecule.

Manners, 1985). Figure 1 shows alternating crystalline (1) and amorphous (2) regions in amylopectin. The linear chains in the molecule are classed as "A" (nonbranched), "B" (branched) and "C" (the single central chain containing a reducing end group). Estimates of the molecular weight of amylopectin begin at 1 million.

Amylopectin comprises 70 to 80% of most cereal starches and is the only starch in waxy genotypes of corn, sorghum, barley, rice and millet. Waxy cereal starches produce gels with unique texture and clarity and have many food and industrial applications. The "A" chains of amylopectin tend to be short and weakly helical and form a weak, reddish-brown clathrate with iodine. Iodine staining can thus be used to distringuish normal and waxy starch. The degree of branching of amylopectin varies between and within species and influences starch properties.

Intermediate or slightly-branched amylose is thought to comprise 5 to 10% of some starches (Whistler et al., 1984; Banks and Greenwood, 1975). It remains poorly characterized due to the difficulty of isolating it. Fractionation studies using liquid chromatography will improve our understanding of differences in branching and molecular size of starches (Kobayashi et al., 1985).

Physical Properties of Starch

Starches exist in highly organized granules in which amylopectin and amylose molecules are held together by hydrogen bonding. Each species produces granules of characteristic size, shape and properties. Starch granules are cold water-insoluble and swell reversibly. Root and tuber starches swell more than cereal starches. In oats and rice, many granules are synthesized within each amyloplast during seed development, resulting in compound starch granules that break apart into small, angular granules. The other cereals, including corn and sorghum, have simple granules. The true density of starch varies from 1.4 to 1.6 g/cm^3. Granule size ranges from submicron to 200 μm or more among species, with smaller variations seen within a given species. Sorghum starch granules range from 2 to 30 μm in diameter, with spherical to polygonal shape. Normal corn and sorghum starch granules are very similar and even a skilled microscopist cannot distinguish between them. Normal and waxy corn starch granules also have a similar appearance, but high-amylose corn (amylomaize) granules are very irregular in shape.

Starch granules rotate the plane of polarized light and exhibit a characteristic shadow ("Maltese cross"), a phenomenon known as birefringence. Polarized light microscopy is routinely used as a tool to identify starches and flours and to measure the loss of organization of starch granules during processing. The apparent intensity of birefringence depends on the thickness, size, shape, molecular structure and orientation of the granule. In general, a starch granule exhibiting birefringence is considered to be in the native state.

Starch Granule Structure

Starch granules are pseudo-crystals that have organized (crystalline) and relatively nonorganized (amorphous) areas. The crystalline, or micellar region is primarily composed of amylopectin. It is resistant to water entry and enzyme attack and responsible for birefringence of the granule. Some adjacent "A" chains are thought to form a double helix in this region. The amorphous region (gel phase) is rich in

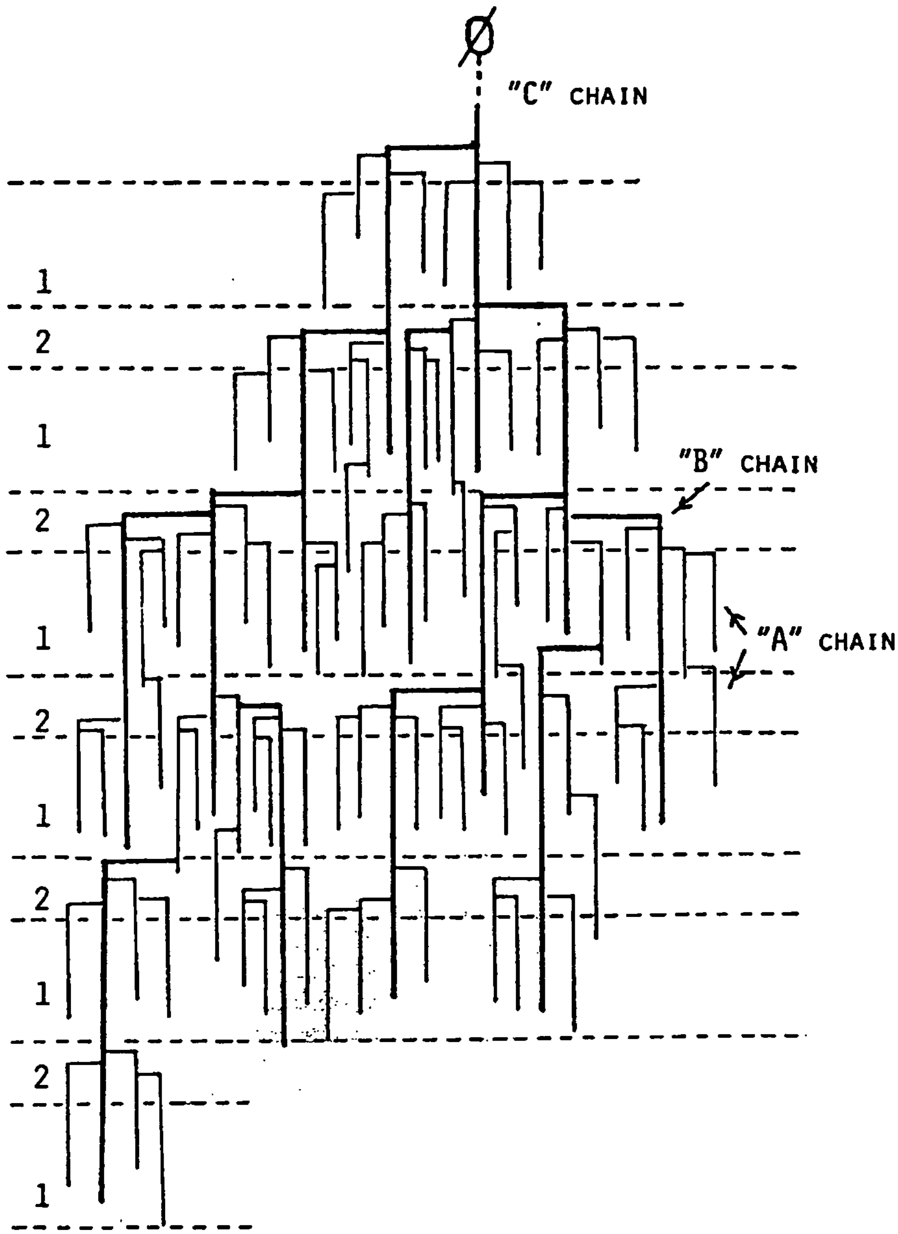

Figure 1. A proposed structure for amylopectin (Manners, 1985).

amylose and less dense than the crystalline area. Water moves freely through it, and amylase attack on the granule begins in this region. Chemical modifications of starch, such as cross-bonding, primarily affect the amorphous area. Current theories (French, 1984; Manners, 1985) describe starch granules as composed of radial chains of amylopectin molecules organized in a semicrystalline cluster arrangement (figure 2). Granule growth occurs concentrically by simultaneous extension of the amylopectin chains. The fact that waxy and normal corn starch granules exhibit similar patterns of enzymatic hydrolysis provides strong evidence that amylopectin is the major structural component of starch granules. Amylase attack begins in the amorphous regions, while hydrolysis of the crystalline regions occurs more slowly.

The exact role of amylose in granule structure is not known. Waxy starches heated in water have much greater swelling power than

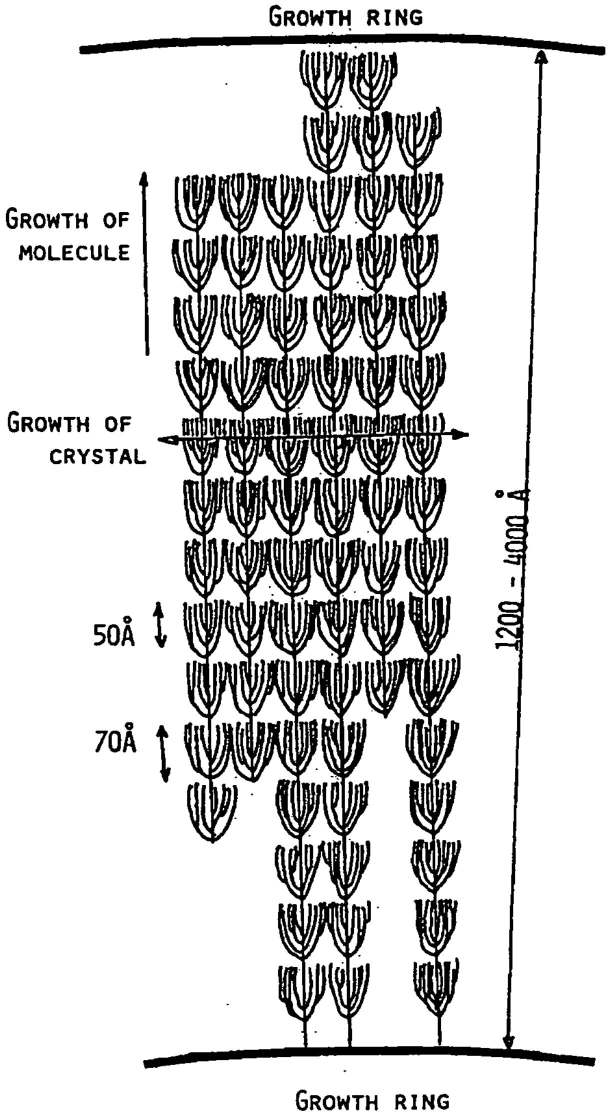

Figure 2. Pattern of crystal growth in a starch granule (French, 1984).

nonwaxy starches, indicating that amylose has a role in restricting granule swelling. It is thought that amylose molecules orient themselves inside the amylopectin crystallites, causing an increase in intermolecular hydrogen bonding which limits both swelling and enzymatic hydrolysis. Another theory maintains that amylose is located primarily in the amorphous regions, where it is complexed with lipids. Amylose is preferentially leached from the starch granule during the early stages of gelatinization, which suggests that at least part of it is in the amorphous areas. These subtle differences in granule structure affect both starch digestibility and the processing properties of grains.

Starch Gelatinization

Starch granules undergo gelatinization, or irreversible loss of native structure, when sufficient energy is applied to break intermolecular hydrogen bonds in the crystalline areas (Lund, 1984; Zobel, 1984). During gelatinization, the starch granules absorb water, swell, exude part of the amylose, become more susceptible to enzyme degradation and lose birefringence. Gelatinization starts in the amorphous areas, but penetration of heat and moisture into the crystalline regions occurs more slowly. It is a semi-cooperative process, in that the stresses generated by swelling of the amorphous areas aid in disrupting the crystallites.

Maurice et al. (1985) have described starch as a partially crystalline, glassy polymer that exhibits nonequilibrium melting behavior. According to this theory, the backbone and branch points of amylopectin constitute the amorphous regions of the granule and the short side chains ("A" chains) provide the crystalline regions. The amorphous areas are thought to melt at a temperature determined by the available water. Water plasticizes the amorphous areas, providing sufficient mobility for the crystallites to melt. Thus, when water is limiting, greater heat or mechanical energy is required to plasticize the amorphous regions and promote loss of organization in the crystallites. This model accounts for the behavior seen in differential scanning calorimetry (DSC) studies of starch and for some common observations in the cooking of starchy materials. Starch gelatinization can be caused by mechanical, thermal or chemical agents or various combinations (Lund, 1984; Zobel, 1984). The presence of free water is of critical importance in starch gelatinization. In high-sugar foods, such as cookies, starch gelatinization is much less than would be expected given the moisture content and baking temperature. Steam-flaking sometimes produces inadequate gelatinization in feed grains, even at high temperatures. Limited free water is the cause of both effects.

Mechanical gelatinization of starch occurs during milling and grinding of cereals. The resulting starch is termed "damaged," a condition that is poorly defined (Evers and Stevens, 1985). Some starch damage is desirable during the milling of wheat into flour. Damaged starch can be attacked by amylases during fermenta-

tion of bread dough to yield the sugars required to sustain yeast activity. Nondamaged starch has low susceptibility to enzyme attack. Dry grinding, rolling and attrition milling produce varying amounts of damaged starch depending on the grain, moisture content and grinding conditions.

Heating of starch in excess water causes starch granules to swell rapidly as intermolecular hydrogen bonds are broken and water becomes bound to starch molecules. As swelling progresses, some amylose leaches from the granule. The characteristic "gelatinization temperature" for a given starch is the point at which it shows 50% loss of birefringence. Near boiling temperature, the granules expand to many times their original size and lose their integrity completely. Dilute alkali and acid promote gelatinization of starch with or without heat.

Heat, moisture, mechanical action and chemical agents are often used in combination to process starchy materials. Steam-flaking involves movement of water and heat into the kernel, causing some swelling of starch. Rolling of the hot, moist grain tears apart some of the swollen granules, forming a paste that binds the other material into a strong flake. The surface area and enzyme susceptibility of the starch are greatly increased by this method. Weak flakes that break easily are produced when insufficient starch "glue" is obtained during steam flaking. Critical factors affecting steam-flaking relate directly to the uptake of water into the endosperm, which permits gelatinization to occur.

Starch Retrogradation and Dextrinization

Retrogradation is the reassociation of starch molecules separated during gelatinization, resulting in the liberation of bound water from the paste or gel. In that sense, it may be viewed as the opposite of gelatinization. Hydrogen bonds between amylose and the "A" chains of amylopectin are reformed, although the retrograded starch does not have the pseudocrystalline character of native starch. The extent of retrogradation depends upon several factors, including the fine structure of amylose and amylopectin, moisture content, temperature, complexing agents (especially lipids), and the concentration of starch in the system. Amylose forms strong retrogrades, stable up to 120 C, while amylopectin retrogrades can be disrupted by gentle heating. Stale bread can be freshened by heating because retrogradation of amylopectin is a major cause of staling. Retrogradation can be beneficial, as in the case of edible amylose films for protecting candies and boil-in-the-bag vegetables. It is more often a disadvantage in foods, as in starch-based pie fillings, which increase in density and release water when retrograded. Starch retrogradation in feeds may decrease digestibility, but probably increases the durability of steam-flaked grains.

Dextrinization may occur during dry-heat processing of grains. Dextrins are fragments of amylose and amylopectin molecules formed by heating dry starch in the presence of some moisture, acids or salts. Dextrinized starch is partially water-soluble and tends to be sticky. Waxy dextrins rehydrate easily and form a strong bond upon drying, making them useful as adhesives. Some unusual, enzyme-resistant glycosidic bonds are formed in starch during dry heat treatment. Processes such as micronizing may thus adversely affect starch digestibility of cereal starches.

Starch Hydrolysis

Amylases hydrolyze starch and are classified according to different criteria including source, products, protein structure, mode of hydrolysis

TABLE 2. STARCH HYDROLASES

Enzyme	Class	Glucosidic bonds attacked	Products
α-Amylases	Liquefying	Endo-α-1,4	Maltose + dextrins
β-Amylases	Saccharifying	Exo-α-1,4	Maltose
Glucoamylases (amyloglucosidases)	Saccharifying	Exo-α1,4 and α-1,6	Glucose
Pullulanases	Debranching	Endo-α-1,6	Linear dextrins
Isoamylases	Debranching	Endo-α-1,6	Linear dextrins

and effects on starch viscosity (Whistler et al., 1984). Amylases of major importance in hydrolysis of starch are summarized in table 2. α-amylases randomly hydrolyze α-1,4 glucosidic bonds within starch molecules, generating maltose and branched and linear dextrins (endo-amylase activity). β-amylases and glucoamylases (amyloglucosidases) attack terminal glucose residues to yield maltose and glucose, respectively (exo-amylase activity). Other starch hydrolases, especially debranching enzymes such as pullulanase, have aided in determining the structure of starch as well as being useful in in vitro digestibility systems. Salivary α-amylase and pancreatic amylases are of critical importance in starch digestion for nonruminants. The linear and branched dextrins produced by α-amylases and debranching enzymes are hydrolyzed much faster by intestinal amyloglucosidases than are larger starch molecules. Thus the yield of glucose from digestion of starchy feeds is increased by their action.

Determination of the Extent of Gelatinization

Estimation of the extent of starch gelatinization is required to monitor the effectiveness of various food and feed processes. Because gelatinization can mean something different to each processor, indexes are often used that relate to important properties of the product. If the index is reproducible and reflects the needs of industry, it can be used effectively.

Viscosity changes, loss of birefringence, paste clarity, changes in swelling and solubility, susceptibility to enzymes and differential scanning calorimetry are methods used to measure gelatinization (Zobel, 1984). Change in viscosity is the most common method of measuring starch gelatinization in the food industry (Lund, 1984).

Birefringence, enzyme susceptiblity of starch and bulk density of flakes (test weight) are indices used in monitoring extent of processing by steam-flaking and micronizing. Test weight determination appears effective as a routine, rapid method of monitoring the consistency of processing during steam-flaking. It is quick, simple, reproducible and requires very little equipment. The bulk density decreases in direct proportion to the thickness of the flakes. The optimum bulk density varies among grains and processors. Steam-flaked sorghum is generally thought to require a lower bulk density than corn or barley for equal feeding value.

The birefringence technique is used as a backup method and for spot-testing of processed feeds. Even in the hands of an experienced microscopist, this method is subject to a great deal of variability. Enzyme susceptibility methods assume that gelatinized starch is rapidly hydrolyzed by enzymes. Thus, the extent of gelatinization is directly related to the amount of sugar produced when the sample is incubated with glucoamylase for a certain time under prescribed conditions. The use of a pure, commercial enzyme gives the most reproducibility in routine enzyme susceptiblity testing. Systems using ruminal fluid are useful for research but are too variable for routine monitoring. Changes in starch viscosity might also be used as the basis for a routine method for measuring gelatinization in feedstuffs.

Factors Affecting the Digestibility of Starches

The digestibility of starch is affected by the composition and physical form of the starch, protein-starch interactions, the cellular integrity of the starch-containing units, antinutritional factors and the physical form of the feed or food material (Thorne et al., 1983; Dreher et al., 1984). A wide variation in digestibility of isolated starch granules in vitro and in vivo has been reported (Dreher et al., 1984). In general, cereal starches are more easily digested than root and tuber starches, while legume starches have intermediate digestibility (table 3). Cooking greatly improves the digestibility of the poorly digested starches. High-amylose corn (amylomaize) has poor digestibility in both raw and cooked forms, while waxy cereal starches are among the most digestible of all starches. Digestibility of a starch is generally inversely proportional to amylose content. Noncooked potato and banana starches both have low digestibility. Interaction with proteins can reduce the susceptibility of both native and processed starch to enzyme hydrolysis. Starch granules can be completely embedded in a protein matrix, as in the corneous and peripheral endosperm of corn and sorghum (figure 3). The effect of the protein matrix on in vitro starch hydrolysis of ground sorghum is illustrated in figure 4. The pronase treatment significantly increased the rate of starch hydrolysis because it hydrolyzed the protein matrix and increased the surface area of the starch in contact with amyloglucosidase. It is also possible for gelatinized starches to form complexes with pro-

TABLE 3. IN VITRO DIGESTIBILITY OF ISOLATED STARCHES

Starch source	Relative susceptibility to amylase attack (normal corn starch = 100) Pancreatic α-amylase[a]	Bacterial α-amylase[b]
Cereal		
Waxy corn		117
Normal corn	100	100
High-amylose corn	45	28
Waxy sorghum		113
Normal sorghum		101
Wheat	100	98
Rice	100	66
Root and tuber		
Potato	7	37
Sweet potato	92	
Arrowroot		42
Tapioca		113
Banana	43	
Legume		
Phaseolus vulgaris (bean)	51	
Faba bean	75	

[a]Moran (1982).

[b]Banks and Greenwood (1975).

teins that reduce digestion of both starch and protein (Thorne et al., 1983). Certain lipids can complex with the amylose in both native and gelatinized starch. The effects on digestibility are not known.

Antinutritional factors affecting starch utilization include enzyme inhibitors, phytates, lectins and tannins (Dreher et al., 1984). The tannins present in brown, bird-resistant sorghums bind proteins and inhibit some enzyme systems and may effectively reduce starch digestion (Hahn et al., 1984).

Feeding Value of Sorghum and Corn

It is well-documented that sorghum must be more vigorously processed than corn, barley or wheat to achieve optimum digestibility of the grain (Rooney and Riggs, 1971; Hale, 1973). Riley (1984) reviewed research trials in which nutritional values of feed grains were compared, and concluded that processed sorghum had about 93 to 96% of the value of corn. The values for wheat and barley were 100% and 97%, respectively, on an equal-weight basis. His conclusions differed from the NRC (1984) values for sorghum, which suggested that sorghum had only 88% the value of corn.

The digestibility of sorghum protein is consistently lower than corn, wheat and barley proteins, a fact that affects ruminal starch digestibility as well (Hale, 1973; Spicer et al., 1982, 1983). The lower digestibility of sorghum protein has been confirmed by Tanksley and co-workers during the past 25 yr with swine feeding and digestion trials (Tanksley and Knabe, 1984). They have found the protein of yellow sorghum to be 5% less digestible than that of corn. The proteins of sorghum are more difficult to extract using classical solvent extraction techniques than any other cereal (Wall and Paulis, 1978). These observations indicate the important role that protein digestibility plays in sorghum feeding value. Although sorghum is fed primarily for its energy value in starch, the endosperm protein matrix must be disrupted if sorghum feeding potential is to be realized. The section on kernel structure will elaborate on this point.

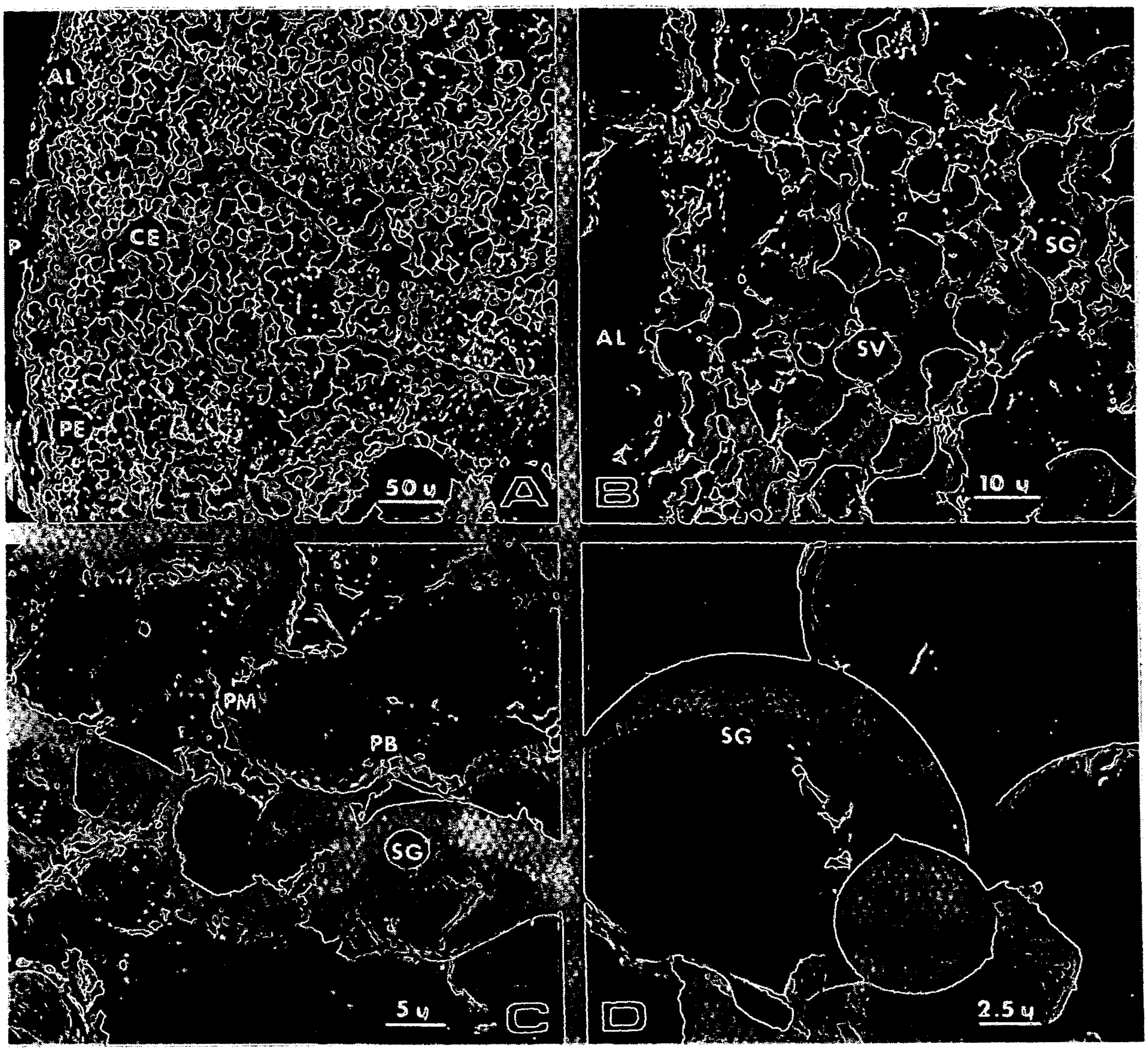

Figure 3. Scanning-electron photomicrographs of intermediate-texture sorghum kernels. A) Endosperm cross section (P = pericarp, AL = aleurone cell layer, PE = peripheral endosperm, CE = corneous endosperm; approx 200X). B) Corneous endosperm area (SV = starch void, SG = starch granule; approx 1,000X). C) Protein and starch of corneous endosperm (PM = protein matrix, PB = protein bodies, SG = starch granule; approx 2,000X). D) Starch of floury endosperm (approx 4,000X).

Digestibility and Feeding Value of Sorghum Types

Sorghum cultivars vary considerably in processing properties and feeding values. Typical feeding values for different sorghum types fed to steers are summarized in table 4; digestibility values are presented in table 5. Brown, bird-resistant sorghums have significantly lower digestibility and produce poorer animal performance than other sorghums (Maxson et al., 1973; Hahn et al., 1984). The heteroyellow endosperm sorghums generally show improved feeding value over the older, nonyellow sorghum cultivars. Waxy sorghum cultivars consistently gave better feed efficiencies than nonwaxy (table 4). These results held in every case with steers, but sheep feeding data were less conclusive. Additional ruminal digestion and "in situ" ruminal digestibility studies indicate that the waxy sorghums have improved ruminal digestibility (Samford et al., 1970; Walker and Lichtenwalner, 1977; Lichtenwalner et al., 1978). The studies presented here have been made using sorghums that have similar kernel

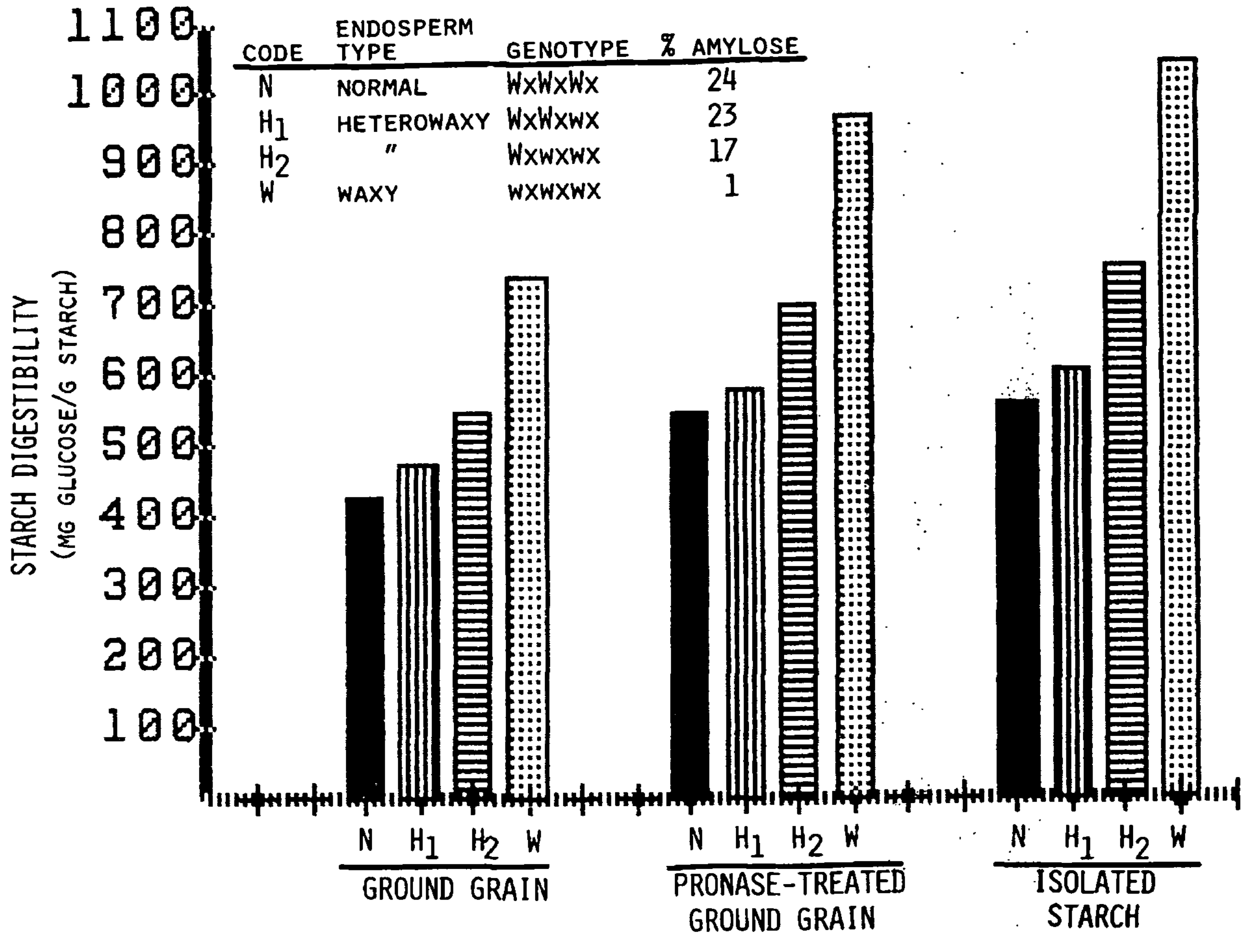

Figure 4. In vitro digestibility of starch from nonwaxy (Wx,Wx,Wx), heterowaxy (Wx,Wx,wx and Wx,wx,wx) and waxy (wx,wx,wx) sorghums (Lichtenwalner et al., 1978).

characteristics, and should provide the most meaningful comparisons. The use of the waxy endosperm trait to improve sorghum feeds appears promising.

Tanksley and Knabe (1984) have compared corn and sorghum feeding values for swine over a 25-yr period, concluding that sorghum cultivars without a pigmented testa have about 95% of the feeding value of yellow dent corn. Brown bird-resistant sorghums had 85% or less of the feeding value of yellow dent corn. They concluded that waxy sorghums average 5% higher in feeding efficiency for swine than nonwaxy sorghums (Cohen and Tanksley, 1973). The waxy grain appears to have consistently higher protein digestibility in the small intestine, which may account for the improved efficiency.

In Vitro and In Vivo Comparisons of Sorghum Varieties

Numerous comparisons of sorghum cultivars have been made using in vitro digestibility methods (Hinders, 1968; Samford et al., 1970; Lichtenwalner et al., 1978; Hahn et al., 1982; Hibberd et al., 1982a,b). Table 6 shows the relative in vitro digestibilities of the ground grain and isolated starches from corn and several sorghum varieties (Wagner, 1984). The in vitro dry matter disappearance (IVDMD) of ground sorghum showed great variation, but all varieties tested were lower than ground corn. Little difference was seen in the IVDMD values for isolated starches, however. Gas production values for the same samples were higher for ground waxy and floury sorghums than for corn, but lower for sorghums with intermediate

TABLE 4. SUMMARY OF FEEDING TRIAL DATA COMPARING WAXY AND NORMAL ENDOSPERM SORGHUM GRAIN DIETS FOR FINISHING STEERS

Study	Variety	Endo-sperm type	Daily gain, kg	Daily feed intake, kg	F/G	Relative F/G
Brethour and Duitsman (1965)[a]	TX09	Normal	1.22		6.72	109
	RS610	Normal	1.33		6.86	111
	Texioca 54	Waxy	1.33		6.16	100
Sherrod et al. (1969)[b]		Normal	1.40	12.47	8.93	107
		Waxy	1.37	11.34	8.28	100
McCollough et al. (1972)[c]	RS671	Normal	.90	7.40	8.35	117
		Heterowaxy	1.01	7.07	6.95	100
		Normal HY[e]	.96		7.94	114
McCollough et al. (1973)[d]	RS671	Normal	1.02	8.47	8.34	113
	CP622	Waxy	1.14	8.29	7.27	100
		Heterowaxy	1.12	9.09	8.08	111
		Heterowaxy	1.07	8.41	7.90	109
		Normal HY[e]	1.10		7.48	103

[a]High grain diet. Waxy feed conversion efficiency not significantly lower.

[b]Steam-rolled, 83.9% grain diet. Waxy feed conversion efficiency lower ($P<.05$).

[c]Dry-rolled, 97.7% grain diet. Waxy feed conversion efficiency lower ($P<.05$).

[d]Dry-rolled, 87.5% grain diet.

[e]HY = heteroyellow endosperm.

and corneous endosperm texture. The low gas production in ground bird-resistant sorghum probably resulted from inactivation of the amyloglucosidase by tannins.

Numerous studies at Texas A&M have found waxy sorghums to have more easily hydrolyzable starch and, to some extent, protein than their nonwaxy counterpart sorghum varieties (figure 5, 6; Sullins and Rooney, 1974, 1975; Lichtenwalner et al., 1978). The kernel characteristics of waxy genotypes greatly affect their properties. Brown, bird-resistant waxy sorghums have poor in vitro starch and protein digestibilities, but considerable variation exists within the brown sorghums depending upon their kernel characteristics. In our studies, no consistent pattern has been established for the in vitro digestibility of nonwaxy sorghums with intermediate endosperm texture. The effect of environmental conditions during maturation of the sorghum kernel is an important determinant of digestibility.

Processing Properties of Sorghums

Sorghum varieties differ widely in their processing properties (Rooney et al., 1980; Rusnak et al., 1980). Considerable variation is seen in the response of sorghum to popping, micronizing, steam-flaking and other feed processing methods. Kernel hardness, kernel size and rate of water uptake are among the important factors. Rusnak et al. (1980) found that waxy sorghum was more thoroughly processed by micronizing than nonwaxy sorghums. Waxy Redlan sorghum produced micronized flakes with a bulk density of 210 g/liter compared with 400 g/liter for nonwaxy Redlan. A similar pattern was seen for several other waxy and heterowaxy genotypes compared to their nonwaxy counterparts. Differences in extent of processing were confirmed by chemical, physical and microscopic analysis. Waxy sorghums were found to expand more during continuous cooking and extrusion than nonwaxy sorghums (Lamar, 1973). Steam-flaking of waxy sor-

TABLE 5. SUMMARY OF DIGESTIBILITY DATA FOR HIGH-CONCENTRATE SORGHUM DIETS FED TO SHEEP AND FINISHING STEERS

Digestibility index	Animal								
	Sheep				Steer				
	CP[a]	NFE[b]	DE[c]	Ref[d]	CP[a]	NFE[b]	DE[c]	GE[e]	Ref[d]
Sorghum type									
Bird-resistant	59.0	84.8	3.2	1	21.5	77.2	2.8	59.7	3
Bird-resistant	61.4	82.6	3.1	1	23.9	60.0			5
Waxy	71.6	87.3	3.4	1	62.1	75.7		66.6	4
Waxy	48.2	82.0	3.0	2	43.5	67.6			
Heterowaxy	68.4	90.4	3.4	1	57.7	84.4	3.3	73.9	3
Heterowaxy	65.6	86.4	3.2	1	51.7	66.7		56.5	4
Normal[f]	71.9	90.4	3.4	1	46.3	76.0	3.1	67.6	3
Normal[f]	70.8	87.4	3.3	1	54.9	68.8		60.5	4
Normal[f]	46.7	78.8	2.8	2	40.4	68.4			5
Heteroyellow	73.4	89.0	3.4	1	51.8	76.9	3.1	66.9	3
Heteroyellow	66.1	85.3	3.2	1	54.9	69.5		60.5	4
Heteroyellow	50.1	82.8	3.0	2	44.7	68.6			5

[a]CP = crude protein digestibility (%).

[b]NFE = nitrogen-free extract (starch) digestibility (%).

[c]DE = digestible energy (kcal/g dry matter).

[d]Ref = references: 1–Sherrod and Albin (1973), 2–Nishimuta et al. (1969), 3–McCollough et al. (1972), 4–McCollough (1973) and 5–McGinty (1968).

[e]GE = gross energy (%).

[f]Non-yellow endosperm.

TABLE 6. RELATIVE IN VITRO DIGESTIBILITY MEASUREMENTS OF WHOLE GRAIN FLOURS AND ISOLATED STARCHES FROM SORGHUM AND CORN[a]

	Gas production[b]				Dry matter disappearance[c]			
	Starch		Flour		Starch		Flour	
Grain	1[d]	2	1	2	1	2	1	2
Corn	100	100	100	100	100	100	100	100
Waxy Redlan	129	126	122	104	100	88	94	91
Redlan	106	113	94	89	89	82	93	88
OK612	110	120	89	82	93	98	74	86
Floury BR	122	122	121	113	102	98	84	85
Bird-resistant	117	121	21	18	87	92	58	51

[a]Adapted from Hibberd et al. (1982a,b).

[b]Volume of gas produced after incubation of sample with amyloglucosidase and yeast for 6 h.

[c]Dry matter disappearance after incubation of sample with buffered ruminal fluid.

[d]Year.

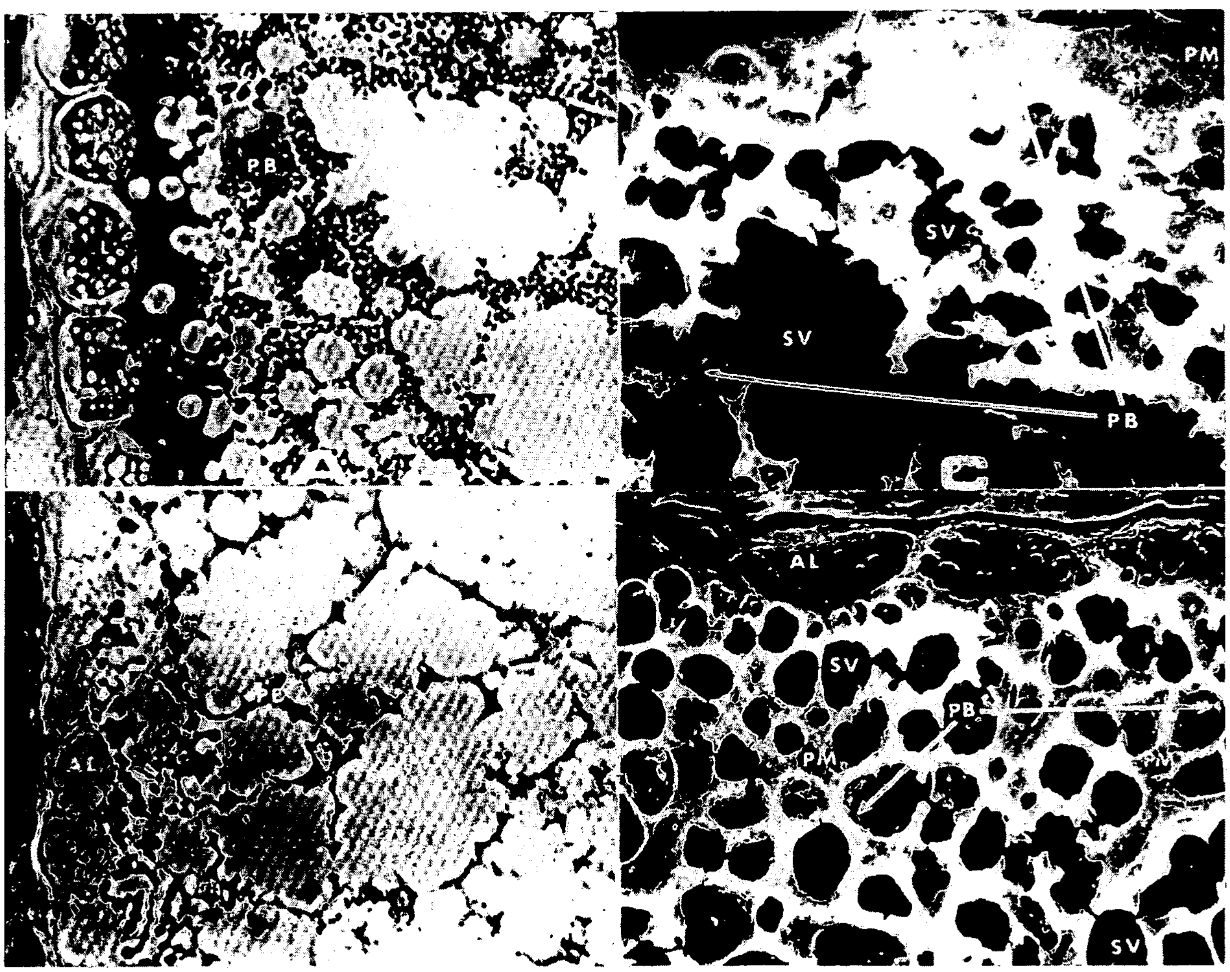

Figure 5. Light and scanning electron photomicrographs of the endosperm structure of nonwaxy and waxy sorghum kernels. A) Nonwaxy kafir, 320X. B) Waxy kafir, 320X. C) Nonwaxy kafir, 1,000X. D) Waxy kafir, 1,000X. Starch removed by α-amylase digestion (AL = aleurone cells, PM = protein matrix, PB = protein bodies, SV = starch voids); (Sullins and Rooney, 1974).

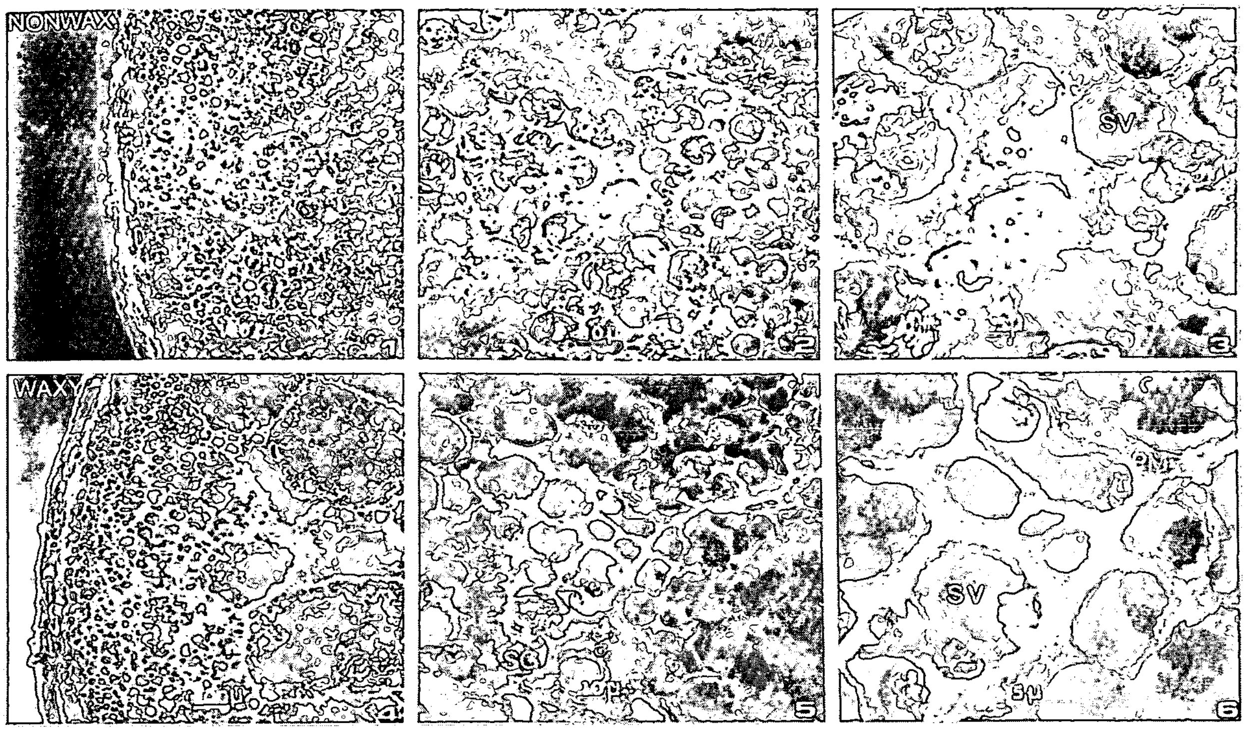

Figure 6. Scanning electron micrographs of nonwaxy and waxy sorghum after 2-h α-amylase digestion at 39 C. Photos 1, 2 and 3: nonwaxy Redlan. Photos 4, 5 and 6: waxy Redlan (SV = starch voids, SG = starch granules, PM = protein matrix, PB = protein bodies, AL = aleurone layer, P = pericarp); (Sullins and Rooney, 1975).

ghums might require much less energy for equal feeding value than nonwaxy sorghums.

Why is Sorghum Less Digestible than Corn?

The gross composition and kernel structure of sorghum and corn are similar (Rooney et al., 1980). Corn has about 1% higher ether extract and sorghum has 1% higher protein content than corn. The starch granules of normal corn and sorghum are very similar in size, shape and composition. Besides kernel size and shape, the major differences between corn and sorghum relate largely to the type and distribution of proteins surrounding the starch in the endosperm. The endosperm of both corn and sorghum is made up of peripheral (sub-aleurone), corneous and floury areas (figure 3). Sorghum generally has a much higher proportion of peripheral endosperm than corn (Rooney and Sullins, 1973; Rooney and Miller, 1982). The peripheral endosperm region is extremely dense, hard and resistant to water penetration and digestion. Peripheral cells have a high protein content and resist both physical and enzymatic degradation. This region also provides some protection to the underlying endosperm cells, which are richer in starch. Low starch yields from wet milling of sorghum were concluded to be due in large part to this layer (Watson et al., 1955; Norris, 1972). Rooney and Riggs (1971) postulated a relationship between starch recovery from wet milling and ruminal digestibility of sorghum, which was also suggested by Wagner (1984). Separation of starch and protein by wet milling is more difficult in sorghum than in corn, and the resulting starch generally contains more protein than commercial corn starch. Significant differences in wet milling properties among sorghum varieties have been documented (Norris and Rooney, 1970; Norris, 1972; Wagner, 1984).

Hardness or corneousness in sorghum and corn is related to protein content and continuity of the protein matrix (Rooney and Miller, 1982). The matrix may be continuous or incomplete and consists of glutelins in which starch granules and prolamine-rich protein bodies are embedded. In corneous endosperm, starch granules are smaller and the matrix nearly continuous. Floury endosperm cells tend to have more and larger starch granules surrounded by a discontinuous matrix with fewer protein bodies. Scattering of light by the num-

erous voids in floury cells makes the floury endosperm opaque.

The protein composition of sorghum and corn endosperm is very similar, but important differences exist (Wall and Paulis, 1978). Intermolecular crosslinks are found in some sorghum prolamines, called cross-linked kafirins. The cross-links decrease the digestibility of both the protein and the starch granules enmeshed in it. Endosperm starch and protein appear to adhere more tightly in sorghum than corn. Some types of cooking have been found to strengthen this starch-protein interaction in sorghum further reducing the rate of starch digestion. Corn endosperm proteins have not been reported to behave similarly.

Higher digestibilities of waxy corn and sorghum appear to relate to differences in the susceptibility of both endosperm protein and starch to digestive enzymes (Sullins and Rooney, 1974, 1975; Walker and Lichtenwalner, 1977; Lichtenwalner et al., 1978). In waxy sorghum, the distribution of protein in corneous endosperm appears more uniform, producing kernels without the pronounced peripheral layers of normal sorghum (figure 5). The protein matrix and protein bodies are thus more easily attacked by proteases, exposing starch granules to amylase attack (figure 6). As discussed earlier, waxy starch granules have been shown to be more susceptible to enzyme hydrolysis than nonwaxy.

Observations on Processing of Sorghum

The requirement of sorghum for effective processing before feeding has been explained in structural, chemical and enzymatic terms in this review. The standard processing methods of steam-flaking, micronizing, popping, reconstitution + grinding, and early harvesting + grinding improve the nutritional value of sorghum to a level which is nearly comparable to corn. The improvement does not derive primarily from starch gelatinization. Both reconstitution and early harvesting improve digestibility without the application of heat intended to gelatinize starch. These effects can be attributed largely to reduced interference with starch hydrolysis by the endosperm protein matrix. Reconstitution causes fermentative degradation of the matrix, while early-harvested grain is utilized before the matrix is completely formed and solidified (Sullins et al., 1971; Hale, 1973). In both cases, starch granules can be separated from the matrix and cell walls during grinding or mastication. Steam-flaking and micronizing cause partial gelatinization of starch and disruption of the endosperm cells by the pressure of flaking. Heat and mechanical force are used to increase the endosperm surface area exposed to digestive enzymes. Therefore, gelatinization measurements are not valid indexes of digestibility improvement for all feed processing methods.

Studies of traditional methods of preparing sorghum foods in Africa and elsewhere tend to confirm the importance of disrupting the peripheral endosperm (Rooney and Murty, 1982). Such treatments as malting, fermentation and cooking with acid or alkali have been shown to modify endosperm cell walls and protein matrix, exposing starch granules to enzyme action.

Water Uptake and Movement in Grain

Steam-flaking and reconstitution require rapid water uptake by grains to achieve complete kernel hydration in minimum time. Water enters kernels by specific routes and at rates that can be altered to some extent by changing treatment conditions (figure 7). Water uptake is determined by the macro- and microstructure, condition and chemical composition of the grain, the temperature, physical state and solute content of the water, and the relative amount

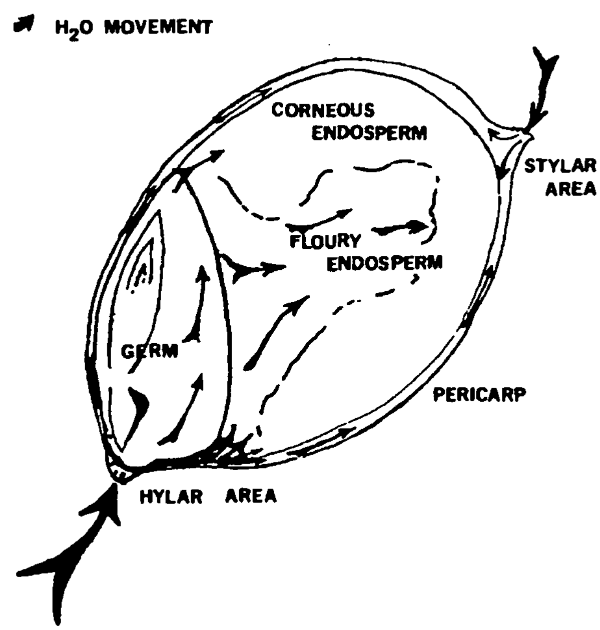

Figure 7. Water uptake and movement in a sorghum kernel (Glueck and Ronney, 1978).

and distribution of the water over the grain surface area. The rate of water uptake can be enhanced effectively by maintaining a high water temperature. The pathways of water movement into corn and sorghum are similar (Wolf et al., 1952; Glueck and Rooney, 1978). Both grains have strong pericarp tissues that prevent water from moving through the intact pericarp. Sorghum has a thicker waxy cuticle on the surface of the epicarp than corn. Water enters at the tipcap of the corn kernel, diffuses through the tube cell layer of the pericarp, and enters the endosperm through the dent region and the germ. In sorghum, water enters at the pedicel near the hilum and at the stylar area, diffusing into the endosperm through the germ, since no floury dent is present. The aleurone layer has thick cell walls that effectively prevent rapid movement of water into the endosperm in both corn and sorghum. Although the peripheral endosperm is very close to the surface of the kernel, it becomes hydrated last, after water has penetrated the germ, floury and corneous endosperm layers.

Conditioning of grain for steam-flaking is essential to produce flakes with low bulk density and high durability. Steam-flaking processes can be improved by modifying conditioning practices to accelerate the normal mechanism of water uptake. The effectiveness of scarification and grain conditioners in enhancing water uptake and processing properties is questionable. Scarification allows water to penetrate the pericarp, but does little to disrupt the aleurone and peripheral endosperm layers. We believe that efforts to develop more efficient conditioning techniques would be productive and profitable in feedlots.

Sorghum Improvement

Sorghum hybrids were developed about 25 yr ago. The utilization of genetic diversity from around the world by aggressive plant breeding programs has led to rapid changes in the type of sorghums grown. The poor quality, brownish-red hybrids of the early 1960s have been replaced by several generations of new hybrids with yellow endosperm, tropical adaptations and other desirable agronomic properties. These rapid changes have made some available information on sorghum composition and nutritional value obsolete. Recent sorghum hybrids have thin red or white pericarp and no pigmented testa. New hybrids with thin white pericarp, tan plant color, yellow endosperm and excellent milling and food quality have been released. Some waxy parental lines are nearing release, so improved heterowaxy and waxy hybrids will likely be forthcoming.

Waxy endoseprm is not expressed fully unless the triploid endosperm is homozygous recessive for the waxy trait (wxwxwx). Grain produced from heterowaxy hybrid sorghum seed contains 25% waxy kernels, 50% heterowaxy kernels (Wxwxwx and WxWxwx) and 25% normal kernels (WxWxWx). The presence of the waxy trait generally improves the feeding value of the grain over normal endosperm varieties. Moreover, the agronomic properties of heterowaxy hybrids are much better than those associated with waxy sorghums. Homozygous waxy sorghums tend to have poorer seedling emergence and vigor and deteriorate more rapidly before harvest than heterowaxy hybrids. Grain yields are generally reduced by the presence of the waxy trait, but commercial heterowaxy hybrids have been successfully grown in the past. Waxy kernels can easily be detected in market channels by use of iodine staining techniques. Thus, livestock producers could contract for waxy or heterowaxy sorghums and be assured of obtaining the high feeding value which these hybrids offer.

The livestock industry can hasten the development of improved sorghum hybrids by their active support and encouragement of sorghum breeding programs. The combination of the waxy and yellow endosperm traits in new sorghum lines with desirable agronomic properties will benefit sorghum and livestock producers alike.

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(54) Title: CHEWING GUM CONTAINING LOW LEVELS OF MALTODEXTRIN

(57) Abstract

A chewing gum composition is disclosed that contains about 0.1 % to about 0.6 % maltodextrin. The maltodextrin provides an initial stiffness so that the gum can be easily wrapped. However, at the low levels used, the maltodextrin does not have a significant impact on the chew properties of the gum.

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CHEWING GUM CONTAINING LOW LEVELS OF MALTODEXTRIN

BACKGROUND OF THE INVENTION

The present invention relates to improved compositions of chewing gum. More particularly, the invention relates to improving the processability of chewing gum by the use of low levels of maltodextrin.

The texture of chewing gum is generally controlled by the amount of moisture it contains and the carbohydrates or polyols used to produce the gum. For sugar gum, the carbohydrates usually come from corn syrup and sugar, and for sugarfree gum, the carbohydrates comprise polyols, such as from sorbitol liquid and hydrogenated starch hydrolyzate (HSH) solutions. The moisture of the gum composition is controlled by varying the level and moisture content of the syrup, sorbitol liquid or HSH solution. Also, texture can be controlled by varying the level of the glycerin softener or other softeners used in the gum. The texture of the gum is also affected by the gum base (soft vs. hard) and flavor type and level (plasticizying vs. non-plasticizing - low vs. high).

In recent years, efforts have been devoted to producing gums that have high flavor levels, and to produce gums with wax-free gum bases. For example the following PCT applications disclose wax-free chewing gums: Application Serial Nos. US93/017580; US93/017578; US93/017579; and US93/017576. The

following U.S. patent applications disclose chewing gum compositions with high flavor levels: Serial Nos. 08/226,667; 08/226,463; 08/226,658; and 08/242,301.

In these newer, more recent gum formulations, the gum base and/or increased flavor levels have had a softening effect on the gum. Consequently, lower levels of glycerin, or moisture containing syrup, sorbitol liquid, or HSH solution would normally be used. However, acceptable lower levels have not giyen gum a sufficiently increased toughness for processing. Further reduction in the level of glycerin or moisture containing syrup, sorbitol liquid or HSH solutions causes gum to be too dry and cracks easily. The low toughness or softness causes problems with the gum as it is fed to high speed wrapping machines.

Maltodextrin is approved for use in food products in the U.S. by the USFDA. U.S. Patent No. 4,604,287 discloses a low moisture chewing gum that contains 0.75% to 6% maltodextrin to produce a gum with an initial soft-short texture which, upon chewing, produces a soft, elastic-cohesive chewing gum.

Maltodextrin is a common encapsulating agent for flavors and high intensity sweeteners. Several patents disclose the use of maltodextrin encapsulated ingredients in chewing gum. For example, U.S. Patent No. 5,139,798 discloses the use of a codried sucralose and maltodextrin mixed with polyvinyl acetate for use in chewing gum.

PCT Publication No. WO 93/5663 discloses the use of ingestible dextrin with aspartame (APM) in chewing gum.

SUMMARY OF THE INVENTION

Unexpectedly, it has been discovered that a low level of maltodextrin added to the gum formulations increases the toughness of the gum to sufficient levels

to improve wrapping efficiency without having a significant effect on the gum's chew characteristics.

In a first aspect, the invention is a chewing gum composition comprising about 5% to about 95% gum base, about 5% to about 95% bulking and sweetening agents, about 0.1% to about 10% flavoring and about 0.1% to about 0.6% maltodextrin.

In a second aspect, the invention is a process for producing a chewing gum comprising the steps of providing gum base comprising about 5% to about 95% of the gum composition, providing bulking and sweetening agents comprising about 5% to about 95% of the gum composition, providing flavoring comprising about 0.1% to about 10% of the gum composition, providing maltodextrin comprising about 0.1% to about 0.6% of the gum composition and combining the gum base, bulking and sweetening agents, flavoring and maltodextrin to form the chewing gum composition.

In a third aspect, the invention is a method of making and wrapping chewing gum sticks comprising the steps of combining gum base, bulking and sweetening agents, flavoring and maltodextrin to form a chewing gum composition comprising about 0.1% to about 0.6% maltodextrin and having a Taber stiffness value of between about 15 and about 40 at 24 hours after mixing, forming the chewing gum composition into sticks, and wrapping the sticks while the Taber stiffness value of the composition is above about 15.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS OF THE INVENTION

As used herein, the term "chewing gum" also includes bubble gum and the like. Unless otherwise specified, all percentages used herein are weight percents.

Maltodextrin is a carbohydrate bulking agent that is low in sweetness intensity. Typical

maltodextrins are made by acid and/or enzyme hydrolysis of starches. They are hydrolyzed to a Dextrose Equivalent (D.E.) of about 4-27 and are typically spray dried to a powder. They are also readily digestible since the glucose polymer consists essentially of α-1,4 bonds between glucose molecules. For this reason, and since maltodextrin also contains some dextrose, maltose, and maltotriose (DP1, DP2, DP3), it could be considered a sugar and will cause dental caries. Thus maltodextrins may preferably be used in sugar type gum formulations.

In order to use maltodextrins in a sugarfree gum, the maltodextrins would have to be treated to remove dextrose, maltose, and maltotriose such as by fermentation, as was disclosed for indigestible dextrin in copending application Serial No. 08/211,197 (based on PCT Publication No. WO 93/5663) and U.S. Patent No. 5,236,719, both incorporated herein by reference. The purified maltodextrin will still remain digestible, and may be hydrolyzed by salivary alpha amylase to fermentable carbohydrates, but it could be used to give reduced dental caries by reducing plaque pH drop. Also, depending on the definition by various countries, purified maltodextrin may be considered sugarfree or carbohydrate modified.

Removal of the fermentable components can be done by yeast fermentation, various types of chromatography, including liquid chromatography and gel permeation chromatography, ultrafiltration, and the use of glucose oxidase and maltase enzyme systems.

The yeast fermentation process is one method of eliminating fermentable components from maltodextrin. The process involves the following steps:

1) Prepare a 20% solution of maltodextrin in water and adjust the pH of the solution to 4-4.5.

2) Add 0.5% Bakers Yeast (by weight of maltodextrin) and stir constantly, at 20° - 35° C. for 4-16 hours or until all the glucose and maltose are gone as analyzed by HPLC.

3) When completed, bring the solution to a boil for 5-10 minutes to inactivate the yeast.

4) Filter out the insoluble portion.

5) Evaporate, freeze dry or spray dry the filtrate.

6) Optionally, the filtrate may be decolorized by treatment with activated carbon and/or treated through an ion-exchange column to remove degraded protein and to deionize the filtrate.

Indigestible dextrin, such as Fibersol from Matsutani, is classified by the USFDA for allowance in food as a maltodextrin because it generally meets the USFDA definition of a maltodextrin. Although it may be considered a maltodextrin, it is not typical of conventional maltodextrins. Although it has a D.E. in the same range (about 4 to 27) as maltodextrin, indigestible dextrin has linkages besides the α-1,4 bonds of maltodextrin, for example α-1,6, β-1,2, β-1,3 and β-1,6. For purpose of the present invention, and as used in the claims, maltodextrin includes indigestible dextrin, such as the indigestible dextrins disclosed in PCT Publication No. WO 93/5663. Also, indigestible dextrin and conventional maltodextrin may together be considered as α-D-glucose polysaccharides.

The use of low levels of maltodextrin in a chewing gum has unexpectedly been shown to offer unique advantages. A low level of maltodextrin added to the gum formula increases the toughness of the gum to sufficient levels to improve wrapping efficiency without having a significant effect on chew characteristics. This level of maltodextrin is about 0.1 to about 0.6% of the gum. Preferred levels are about 0.2 to about 0.5% maltodextrin in gum.

According to U.S. Patent No. 4,604,287, maltodextrin used at a level of about 0.75% and above gives gum an initial non-cohesive (short) texture. However, preferred gums of the present invention have an initial soft, cohesive texture.

Any maltodextrin may be used that is a dry powder (less than 6% moisture) and has a D.E. in the range of about 4-27. The preferred D.E. range is about 7-24, and the most preferred D.E. is in the range of about 10-17.

Recent advances use hydrogenated starch hydrolyzates (HSH) and glycerin preblended and co-evaporated to reduce moisture in some sugar-free gum formulations. Maltodextrin may be used in gum formulations with hydrogenated starch hydrolyzates (HSH) without preblending with glycerin and coevaporation. The increased toughness of the inventive formulation reduces the need to keep moisture at a minimum.

As noted earlier, maltodextrin is often used as an encapsulating or agglomerating agent. Maltodextrin may also be used to absorb other ingredients. Maltodextrin may be able to encapsulate, agglomerate or entrap/absorb flavors and high-intensity sweeteners like aspartame, alitame, cyclamic acid and its salts, saccharin acid and its salts, acesulfame and its salts, sucralose, dihydrochalcones, thaumatin, monellin or combinations thereof. Encapsulation of high-intensity sweeteners with maltodextrin may improve the sweetener's shelf-life. These encapsulated flavors and/or sweeteners may be added to chewing gum. It is believed that the maltodextrin used in such encapsulations, if used at the proper levels, may provide the initial toughening desired for easy wrappability. However, in the preferred embodiment, the maltodextrin added to provide stiffness is in a

form where it is not bound with any other gum ingredient.

The maltodextrin may readily be incorporated into a chewing gum composition. The remainder of the chewing gum ingredients are noncritical to the present invention. That is, the maltodextrin can be incorporated into chewing gum formulations in a conventional manner. As noted above, however, typically the invention will be utilized in conjunction with soft gum bases and soft gum formulations, especially with high flavor levels.

In general, a chewing gum composition typically comprises a water-soluble bulk portion, a water-insoluble chewable gum base portion and typically water-insoluble flavoring agents. The water-soluble portion dissipates with a portion of the flavoring agent over a period of time during chewing. The gum base portion is retained in the mouth throughout the chew.

The insoluble gum base generally comprises elastomers, resins, fats and oils, waxes, softeners and inorganic fillers. Elastomers may include polyisobutylene, isobutylene-isoprene copolymer and styrene butadiene rubber, as well as natural latexes such as chicle. Resins include polyvinyl acetate and terpene resins. Fats and oils may also be included in the gum base, including tallow, hydrogenated and partially hydrogenated vegetable oils, and cocoa butter. Commonly employed waxes include paraffin, microcrystalline and natural waxes such as beeswax and carnauba. According to the preferred embodiment of the present invention, the insoluble gum base constitutes between about 5 to about 95% of the gum. More preferably the insoluble gum base comprises between 10 and 50% of the gum and most preferably about 20 to about 35% of the gum.

The gum base typically also includes a filler component. The filler component may be calcium carbonate, magnesium carbonate, talc, dicalcium phosphate or the like. The filler may constitute between about 5 and about 60% of the gum base. Preferably, the filler comprises about 5 to about 50% of the gum base.

Gum bases typically also contain softeners, including glycerol monostearate and glycerol triacetate. Further, gum bases may also contain optional ingredients such as antioxidants, colors, and emulsifiers. The present invention contemplates employing any commercially acceptable gum base.

The water-soluble portion of the chewing gum may further comprise softeners, sweeteners, flavoring agents and combinations thereof. The sweeteners often fill the role of bulking agents in the gum. The bulking agents generally comprise from about 5% to about 95%, preferably from about 20% to about 80%, and most preferably from about 30% to about 60% of the gum.

Softeners are added to the chewing gum in order to optimize the chewability and mouth feel of the gum. Softeners, also known in the art as plasticizers or plasticizing agents, generally constitute between about 0.5 to about 15% of the chewing gum. Softeners contemplated by the present invention include glycerin, lecithin and combinations thereof. Further, aqueous sweetener solutions such as those containing sorbitol, hydrogenated starch hydrolyzates, corn syrup and combinations thereof may be used as softeners and binding agents in gum.

As mentioned above, the maltodextrin of the present invention may be used in sugar gum formulations. However, sugar-free formulations are also within the scope of the invention. Sugar sweeteners generally include saccharide-containing components commonly known in the chewing gum art which

comprise, but are not limited to, sucrose, dextrose, maltose, dextrin, dried invert sugar, fructose, levulose, galactose, corn syrup solids and the like, alone or in any combination.

The maltodextrin of the present invention can also be used with aspartame or other high-intensity sweeteners, as well as commonly known sugarless sweeteners. Generally sugarless sweeteners include components with sweetening characteristics but which are devoid of the commonly known sugars and comprise, but are not limited to, sugar alcohols such as sorbitol, mannitol, xylitol, hydrogenated starch hydrolyzates, maltitol and the like, alone or in any combination.

Depending on the particular sweetness release profile and shelf-stability needed, the maltodextrin of the present invention can also be used in combination with coated or uncoated high-intensity sweeteners, such as acesulfame K, or the salts of acesulfame, cyclamate and its salts, saccharin and its salts, alitame, sucralose, thaumatin, monellin, dihydrochalcones, stevioside, glycyrrhizin and combinations thereof.

High-intensity sweeteners may also be modified to control their release in chewing gum formulations containing maltodextrin. This can be controlled by various methods of encapsulation, agglomeration, absorption, or a combination of methods to obtain either a fast or slow release of the sweetener. Sweetener combinations, some of which may be synergistic, may also be included in the gum formulations containing maltodextrin.

A flavoring agent may be present in the chewing gum in an amount within the range of from about 0.1 to about 10%, and preferably, for high flavor level gums, from about 1.5 to about 4% of the gum. The flavoring agents may comprise essential oils, synthetic flavors, or mixture thereof including, but not limited

to, oils derived from plants and fruits such as citrus oils, fruit essences, peppermint oil, spearmint oil, clove oil, oil of wintergreen, anise, and the like. Artificial flavoring components are also contemplated for use in gums of the present invention. Those skilled in the art will recognize that natural and artificial flavoring agents may be combined in any sensorially acceptable blend. All such flavors and flavor blends are contemplated by the present invention.

Optional ingredients such as colors, emulsifiers and pharmaceutical agents may be added to the chewing gum.

In general, chewing gum is manufactured by sequentially adding the various chewing gum ingredients to a commercially available mixer known in the art. After the ingredients have been thoroughly mixed, the gum mass is discharged from the mixer and shaped into the desired form such as by rolling into sheets and cutting into sticks, extruding into chunks or casting into pellets.

Generally, the ingredients are mixed by first melting the gum base and adding it to the running mixer. The base may also be melted in the mixer itself. Color or emulsifiers may also be added at this time. A softener such as glycerin may also be added at this time, along with any syrup and a portion of the bulking agent/sweetener. Further portions of the bulking agent/sweetener may then be added to the mixer. A flavoring agent is typically added with the final portion of the bulking agent. High-intensity sweeteners are preferably added after the final portion of bulking agent and flavor have been added.

The entire mixing procedure typically takes from five to fifteen minutes, but longer mixing times may sometimes be required. Those skilled in the art

will recognize that many variations of the above described procedure may be followed.

Although it is preferable to use maltodextrin in a sugar gum, and a purified maltodextrin in a sugarless gum formulation, either may be used in a gum formula at levels sufficient to provide initial stiffness.

Taber stiffness is a measurement of gum stiffness using a Taber V-5 Stiffness Tester, available from the Taber Instrument Corporation, North Tonawanda, New York, with the gum at room temperature. The higher the Taber value, the tougher the gum. Gums that have a Taber value of about 0-8 are very difficult to wrap, gums with a Taber value of 8-15 are moderately difficult to wrap, gums with Taber value of about 15-20 are moderately easy to wrap, and gums with a value of 20-40 wrap very easily. A preferred Taber stiffness value is in the range of about 25-30 at 24 hours after making the gum.

To demonstrate the invention, five sugar gum formulas were made with a wax-free gum base as follows:

	%
Butyl elastomer (isoprene-isobutylene copolymer)	8.1
Polyisobutylene	6.9
Polyvinyl acetate	21.7
Glycerol esters of hydrogenated rosin	10.5
Terpene resin	13.7
Hydrogenated vegetable oil	13.4
Glycerol monostearate	4.6
Lecithin	1.7
Calcium carbonate	19.1
Color	0.3
	100.0

The following examples used a maltodextrin having a D.E. of 17, sold under the tradename LODEX 15, available from American Maize Products Co.

COMPARATIVE EXAMPLE A AND EXAMPLES 1-2

The following laboratory gum formulations were made:

	Comparative Ex. A	Ex. 1	Ex. 2
No Wax Base	20.90	20.90	20.90
Sugar	62.76	62.51	62.26
Corn Syrup (85% Solids)	14.00	14.00	14.00
Glycerin	0.76	0.76	0.76
Cinnamon Flavor	1.40	1.40	1.40
Color	0.18	0.18	0.18
Lodex 15 Maltodextrin	-	0.25	0.50
	100.00	100.00	100.00

After 7 days, five samples of each formula were tested in a Taber Stiffness Tester. The average Taber stiffness value for the formulas were as follows:

Comparative Example A	16.6
Example 1	22.2
Example 2	25.6

Even though these tests were not conducted at 24 hours after mixing, and the Taber value may have changed during storage, it is expected from the similarities of the formulations and the fact that all samples were stored under the same conditions, that the Taber values at 24 hours would have shown the same increase in Taber stiffness due to the addition of low levels of maltodextrin as was evidenced by the 7-day testing.

COMPARATIVE EXAMPLE B AND EXAMPLE 3

In the next formulations, gum was made on a production scale. Two batches of the inventive formula of Example 3 were made.

	Comparative Ex. B	Ex. 3
No Wax Base	20.40	20.90
Sugar	62.71	62.26
Corn Syrup (85% Solids)	14.50	14.00
Glycerin	0.76	0.76
Cinnamon Flavor	1.45	1.40
Color	0.18	0.18
Lodex 15 Maltodextrin	--	0.50
	100.00	100.00
Average Taber Stiffness (N=5) (24 Hrs.)	16.7	38.9 (Batch 1) 27.4 (Batch 2)

The comparative formulation, besides being slightly too soft for wrapping, was also considered very dry, scaly, and crumbly and caused problems in sheeting. There was also some difficulty in wrapping the comparative gum, but the inventive formulation, Example 3, wrapped very easily. It is not known why the two batches of Example 3 different in Taber stiffness. This variation is not normal. It could be due to cold start up if this batch with Taber at 38.9 was first. In either case, both batches have much higher Taber than Comparative Example B.

COMPARATIVE EXAMPLES C-D AND EXAMPLES 4-7

The following base formulation was used in these comparative and inventive examples, as well as in Comparative Examples E-F and Examples 8-9:

	%
Isobutylene-isoprene copolymer	6.1
Polyisobutylene	4.9
Terpene resins	12.5
High MW (55,000-80,000) polyvinyl acetate	6.6
Medium MW (25,000-55,000) polyvinyl acetate	11.9
Low MW (10,000-25,000) polyvinyl acetate	15.3
Glycerol esters of hydrogenated rosin	5.9
Glycerol monostearate	8.0
Hydrogenated vegetable oil	1.2
Lecithin	3.0
Triacetin	0.9
Calcium carbonate	12.1
Talc	11.1
Color	0.5
	100.0

The following laboratory gum formulations were made:

	Comparative Ex. C	Ex. 4	Ex. 5	Comparative Ex. D	Ex. 6	Ex. 7
Base	26.0	26.0	26.0	26.0	26.0	26.0
Sugar	61.85	61.65	61.65	64.35	64.15	64.15
Corn Syrup (80% solids)	7.1	7.1	7.1	5.0	5.0	5.0
Glycerin	0.9	0.9	0.9	0.5	0.5	0.5
Fruit Flavor	2.45	2.45	2.45	2.45	2.45	2.45
Encapsulated Sweeteners	1.7	1.7	1.7	1.7	1.7	1.7
Lodex 10 *	---	0.20	---	---	0.20	---
Lodex 15 **	---	---	0.20	---	---	0.20

*Lodex 10 is a 10 D.E. maltodextrin from American Maize Co.
**Lodex 15 is a 15 D.E. maltodextrin from American Maize Co.

Based on sensory evaluation of the samples, compared to Comparative Example C, both Examples 4 and 5 were slightly tougher, but gave good quality gum. Similarly, both Examples 6 and 7 were drier and more crumbly than Examples 4 and 5 on a laboratory scale. However, as seen in the next examples, gum formulations with 5% syrup gave soft products on a production scale, requiring the use of maltodextrin to toughen the formulations.

COMPARATIVE EXAMPLES E-F AND EXAMPLES 8-9

The following fruit gum samples were made to evaluate the use of maltodextrin in these type of gum formulations on a production scale.

	Comparative Ex. E	Comparative Ex. F	Ex. 8	Ex. 9
Base	26.00	26.00	26.00	26.00
Sugar	63.25	63.40	64.20	64.35
Corn Syrup (80% Solids)	5.00	5.00	5.00	5.00
Glycerin	0.50	0.50	0.50	0.50
Fruit Flavor	2.55	2.55	2.35	2.35
Calcium Carbonate	1.00	1.00	--	--
Encapsulated Sweetener	1.25	1.25	1.25	1.25
Encapsulated APM	0.45	--	0.45	--
Encapsulated acesulfame K	--	0.30	--	0.30
Lodex 15 Maltodextrin	--	--	0.25	0.25
	100.00	100.00	100.00	100.00
Average Taber Stiffness (N=10) (24 Hrs.)	7.9	7.4	17.1	16.9

In these samples, calcium carbonate was eliminated from the inventive examples and the flavor level was slightly lower than the comparative examples. However, these differences are considered slight compared to the addition of 0.25% maltodextrin. The addition of maltodextrin increases the stiffness of the gum from a product very difficult to wrap to a product that processes more easily and can be wrapped moderately easily.

It is believed that beside maltodextrin, low levels of natural carbohydrate gums such as guar gum, cellulose derivatives such as carboxy methyl cellulose and hydroxypropyl methyl cellulose, gelatine and

modified starches may be used to provide an initial stiffness without affecting the chew properties of the gum.

It should be appreciated that the compositions and methods of the present invention are capable of being exhibited in the form of a variety of embodiments, only a few of which have been illustrated and described above. The invention may be embodied in other forms without departing from its spirit or essential characteristics. It will be appreciated that the addition of some other ingredients, process steps, materials or components not specifically included may have an adverse impact on the present invention. The best mode of the invention may therefore exclude ingredients, process steps, materials or components other than those listed above for inclusion or use in the invention. However, the described embodiments are to be considered in all respects only as illustrative and not restrictive, and the scope of the invention, therefore, is indicated by the appended claims rather than by the foregoing description. All changes which come within the meaning and range of equivalency of the claims are to be embraced within their scope.

WE CLAIM:

1. A chewing gum composition comprising:

a) about 5% to about 95% gum base;

b) about 5% to about 95% bulking and sweetening agents;

c) about 0.1% to about 10% flavoring; and

d) about 0.1% to about 0.6% maltodextrin.

2. The chewing gum composition of claim 1 wherein the maltodextrin has a D.E. in the range of about 4 to 27.

3. the chewing gum composition of claim 1 wherein the maltodextrin has a D.E. in the range of about 7 to 24.

4. The chewing gum composition of claim 1 wherein the maltodextrin comprises about 0.2% to about 0.5% of the gum composition.

5. The chewing gum composition of claim 1 wherein the gum base is free of wax.

6. The chewing gum composition of claim 1 wherein the flavoring comprises about 1.5% to about 4% of the gum composition.

7. The chewing gum composition of claim 1 wherein the flavoring comprises a fruit flavor.

8. The chewing gum composition of claim 1 wherein the maltodextrin has a D.E. of between about 10 and about 17.

9. The chewing gum composition of claim 1 wherein the maltodextrin is freely admixed into the gum and is not bound with any other gum ingredient.

10. The chewing gum composition of claim 1 wherein the gum composition has a Taber stiffness value of between about 15 and about 40 at 24 hours after mixing.

11. The chewing gum composition of claim 1 wherein the composition has an initial soft, cohesive texture.

12. A process for producing a chewing gum comprising the steps of:

a) providing gum base comprising about 5% to about 95% of the gum composition;

b) providing bulking and sweetening agents comprising about 5% to about 95% of the gum composition;

c) providing flavoring comprising about 0.1% to about 10% of the gum composition;

d) providing maltodextrin comprising about 0.1% to about 0.6% of the gum composition; and

e) combining the gum base, bulking and sweetening agents, flavoring and maltodextrin to form the chewing gum composition.

13. The process of claim 12 wherein the maltodextrin is provided in a powdered form and mixed as a powder with the other chewing gum composition ingredients.

14. The process of claim 12 wherein the maltodextrin has a D.E. of between about 4 and about 27.

15. The process of claim 12 wherein the maltodextrin has a D.E. of between about 10 and about 17.

16. The process of claim 12 wherein the maltodextrin comprises about 0.2% to about 0.5% of the gum composition.

17. The process of claim 12 wherein the maltodextrin comprises indigestible dextrin.

18. The process of claim 12 wherein the maltodextrin comprises D-glucose units linked essentially by α-1,4 bonds.

19. A method of making and wrapping chewing gum sticks comprising the steps of:

a) combining gum base, bulking and sweetening agents, flavoring and maltodextrin to form a chewing gum composition comprising about 0.1% to about 0.6% maltodextrin and having a Taber stiffness value of between about 15 and about 40 at 24 hours after mixing;

b) forming said chewing gum composition into sticks; and

c) wrapping said sticks while the Taber stiffness value of the composition is above about 15.

20. The method of claim 19 wherein the composition comprises about 0.2% to about 0.5% maltodextrin.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/09039

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :A23G 3/30
US CL :426/3
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 426/3, 4, 5, 6, 658

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS
search terms: chewing gum, maltodextrin

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US, A, 4,208,431 (FRIELLO ET AL) 17 June 1980, see paragraph bridging cols 5 and 6.	1 ---- 2-20
Y	US, A, 4,238,475 (WITZEL ET AL) 09 December 1980, see examples 3 and 4.	1-20
Y	US, A, 4,590,075 (WEI ET AL) 20 May 1986, see tables.	1-20
Y	US, A, 4, 604,287 (GLASS ET AL) 05 August 1986, see abstract, tables and claims.	1-20
Y	US, A, 4,579,738 (CHERUKURI ET AL) 01 April 1986, see entire document, particularly col 5 lns 43+.	1-20

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
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"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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Date of the actual completion of the international search
20 SEPTEMBER 1995

Date of mailing of the international search report
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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,497,832 (CHERUKURI ET AL) 05 February 1985, see tables.	1-20

:ONTRIBUTORS

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part) number are to be underlined for italics, but
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NEW ZEALAND
JOURNAL OF SCIENCE

Department of Scientific and Industrial Research, Wellington.

Editor: Mabel Rice

VOLUME 5 DECEMBER 1962 NUMBER 4

THE INADEQUACY OF THE USUAL DETERMINATIVE TESTS FOR THE IDENTIFICATION OF *Xanthomonas* SPP.

By D. W. DYE, Plant Diseases Division, Department of Scientific and Industrial Research, Auckland

(Received for publication, 4 September 1962)

Summary

A comparative study has been made of 209 phytopathogenic *Xanthomonas* cultures comprising 57 recognised species, using the so called standard methods in an attempt to clarify the identification of the species by laboratory procedures. The various species that have been proposed could not be differentiated by any or all of the 30 different tests used. They formed a remarkably uniform group which could easily be distinguished from some other yellow pigment producing organisms that were included for comparative purposes. It is suggested that the many *Xanthomonas* species could well be regarded as special forms of one species adapted to particular hosts.

INTRODUCTION

In recent years there has been a steady increase in the number of species identified as *Xanthomonas* but no critical comparison of the many organisms has been published. Lists of cultural and biochemical characters are available (Elliott 1951, Stapp 1956, Bergey 1957, Dowson 1957) but these lists are inconsistent, and give rise to doubt concerning the validity of the differences listed; some may represent no more than natural variation among strains, while others could be due to differences in methods of testing, or of recording results used by different workers. On the other hand some differences that have been listed are so great—e.g., both aerobes and facultative anaerobes have been described—that if they are valid, the status of the genus could be challenged. While there is a growing belief among plant bacteriologists and pathologists that not all species in *Xanthomonas* have been appropriately classified and that the genus should be circumscribed more definitely, in other fields, e.g., soil and industrial microbiology, there

is a tendency to include various yellow pigment producing organisms which can fulfil the present definition of *Xanthomonas* (Turner, 1954; Holding, 1960; Stout, 1961; Brisbane and Rovira, 1961). Bacteriologists and pathologists are therefore seriously hampered by this lack of precise characterisation which is rapidly leading to confusion in the identification and classification of the organisms.

This paper describes the results of a study of cultures all of which were either received as named species of *Xanthomonas* or were isolated and identified by the writer. The tests used were deliberately chosen as being the old or "standard" tests since it is by these methods that the species had been examined when originally identified. Some modifications have, however, been made to suit the particular requirements of the organisms.

CULTURES TESTED

Cultures were received from Dr D. P. Bhide, Poona, India; Dr E. Billing, Wye, England; Professor W. H. Burkholder, Cornell University, U.S.A.; Dr W. J. Dowson, Cambridge, England; Dr W. A. F. Hagborg, Winnipeg, Canada; Dr E. Hellmers, Copenhagen, Denmark; Dr M. K. Hingorani, New Delhi, India; Mr R. A. Lelliott, National Collection of Plant Pathogenic Bacteria, Harpenden, England; Professor H. Muko, Tokyo, Japan; Dr R. Mushin, Melbourne, Australia; Professor H. Okabe, Shizuoka, Japan; Professor Orillo, Laguna, Philippines; Dr A. M. Paton and Dr J. Holding, Edinburgh, Scotland; Dr A. F. G. Slootweg, Lisse, Holland; Professor M. P. Starr, Davis, U.S.A.; Dr R. S. Vasudeva, Indian Type Culture Collection, New Delhi, India. Other cultures were isolated by the author.

The following list gives the cultures (with name as received), the number of strains tested of each organism and the code letters used:

Xanthomonas alfalfae (Riker *et al.*, 1935) Dowson, 1943, ZU1; *X. amaranthicola* Patel *et al.*, 1952, YO1; *X. axonopodis* Starr & Garces, 1950, ZB1; *X. badrii* Patel *et al.*, 1950, XB1; *X. barbarae*, Burkholder 1941, YP2, YP4; *X. begoniae* (Takimoto, 1934) Dowson, 1939, Z1–Z3; *X. beticola* (Smith *et al.*, 1911) Savulescu, 1947, ZV3; *X. betlicola* Patel *et al.*, 1951, YC1; *X. campestris* (Pammel, 1895) Dowson, 1939, Q1, Q2, Q5–Q7, Q9, Q11–Q25; *X. campestris* var. *armoraciae* (McCulloch, 1929) Starr & Burkholder, 1942, Q10; *X. carotae* (Kendrick, 1934) Dowson, 1939, ZC1–ZC4; *X. cajani* Kulkarni *et al.*, 1950, YR1; *X. cassava* Wiehe & Dowson, 1953, ZD1; *X. cassiae* Kulkarni *et al.*, 1951, YD1; *X. citri* (Hasse, 1915) Dowson, 1939, R1–R6, R8–R15; *X. clerodendri* Patel *et al.*, 1952, YS1; *X. corylina* (Miller *et al.*, 1940) Starr & Burkholder, 1942, YQ1, YQ2; *X. cucurbitae* (Bryan, 1926) Dowson, 1939, ZE1, ZE2; *X. desmodii* Uppal & Patel, 1949, YE1, YE2; *X. desmodiigangeticii* Uppal *et al.*, 1948, XE1, XE2; *X. dieffenbachiae* (McCulloch & Pirone, 1939) Dowson, 1943, XD1; *X. erythrinae* Patel *et al.*, 1952, YT1, YT2; *X. geranii* (Burkholder, 1937) Dowson, 1939, YB1, YB2; *X. hederae* (Arnaud, 1920) Dowson, 1939, YV1–YV3; *X. holcicola* (Elliott, 1930) Starr & Burkholder, 1942, YW1; *X. hyacinthi* (Wakker, 1883) Dowson, 1939, Y2, Y5–Y7; *X. incanae*

(Kendrick & Baker, 1942) St
(Pierce, 1901) Dowson, 193
& Anderson, 1937) Savulescu,
YN1; *X. lespedezae* (Ayers
foliigardeniae (Ark, 1946) E
cearum (Smith, 1901) Dowso
hotis (Arthaud-Berthet, 1912
Moniz & Patel, 1958, XG1;
YF1, YF2; *X. nigromacular*
X. nigromaculans f. sp. *zinnia*
(Uyeda & Ishiyama, 1926)
(Bryan & McWhorter, 1930
(Brown, 1923) Starr & Burkh
(Smith, 1897) Dowson, 1939,
holder, 1930) Starr & Burk
sojense (Hedges, 1922) Starr
dicola Goto & Okabe, 1958,
X. poinsettiaecola Patel *et al.*,
1939, T1, T2, T6–T15; *X. pu*
X. ricinicola (Elliott, 1930)
Patel *et al.*, 1952, YH1, YH
X. taraxaci Niederhauser, 194
Dowson, 1939, ZQ1–ZQ15;
X. uppalii Patel, 1948, ZR1–
1939, ZS1–ZS9; *X. vesicatoria*
X7–X10, X12–X14; *X. vesic*
Burkholder 1942, X11; *X.*
X. vitians (Brown, 1918) Dows
Moniz & Patel, 1958, XH1.

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EXPER

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of a study of cultures all of which were
of *Xanthomonas* or were isolated and
used were deliberately chosen as being
it is by these methods that the species
lly identified. Some modifications have,
particular requirements of the organisms.

URES TESTED

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ners, Copenhagen, Denmark; Dr M. K.
Mr R. A. Lelliott, National Collection of
penden, England; Professor H. Muko,
elbourne, Australia; Professor H. Okabe,
o, Laguna, Philippines; Dr A. M. Paton
Scotland; Dr A. F. G. Slootweg, Lisse,
Davis, U.S.A.; Dr R. S. Vasudeva, Indian
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al., 1935) Dowson, 1943, ZU1; *X. amar-*
. *X. axonopodis* Starr & Garces, 1950, ZB1;
; *X. barbarae*, Burkholder 1941, YP2, YP4;
Dowson, 1939, Z1–Z3; *X. beticola* (Smith
V3; *X. betlicola* Patel *et al.*, 1951, YC1;
Dowson, 1939, Q1, Q2, Q5–Q7, Q9,
moraciae (McCulloch, 1929) Starr & Burk-
Kendrick, 1934) Dowson, 1939, ZC1–ZC4;
YR1; *X. cassavae* Wiehe & Dowson, 1953,
1951, YD1; *X. citri* (Hasse, 1915) Dow-
X. clerodendri Patel *et al.*, 1952, YS1;
) Starr & Burkholder, 1942, YQ1, YQ2;
Dowson, 1939, ZE1, ZE2; *X. desmodii*
; *X. desmodiigangeticii* Uppal *et al.*, 1948,
McCulloch & Pirone, 1939) Dowson, 1943,
1952, YT1, YT2; *X. geranii* (Burkholder,
B2; *X. hederae* (Arnaud, 1920) Dowson,
(Elliott, 1930) Starr & Burkholder, 1942,
883) Dowson, 1939, Y2, Y5–Y7; *X. incanae*

(Kendrick & Baker, 1942) Starr & Weiss, 1943, YX1, YX2; *X. juglandis* (Pierce, 1901) Dowson, 1939, S1–S13; *X. lactucae-scariolae* (Thornberry & Anderson, 1937) Savulescu, 1947, ZF1; *X. lawsoniae* Patel *et al.*, 1951, YN1; *X. lespedezae* (Ayers *et al.*, 1939) Starr, 1946, YL1; *X. maculifoliigardeniae* (Ark, 1946) Elrod & Braun, 1947, YM1, YM2; *X. malvacearum* (Smith, 1901) Dowson, 1939, ZG1–ZG7; ZG11–ZG13; *X. manihotis* (Arthaud-Berthet, 1912) Starr, 1946, ZH1–ZH3; *X. martinicola* Moniz & Patel, 1958, XG1; *X. nakatae* (Okabe, 1933) Dowson, 1943, YF1, YF2; *X. nigromaculans* (Takimoto, 1927) Dowson, 1943 ZT2; *X. nigromaculans* f. sp. *zinnae* Hopkins & Dowson, 1949, ZT1; *X. oryzae* (Uyeda & Ishiyama, 1926) Dowson, 1943, YK1–YK5; *X. papavericola* (Bryan & McWhorter, 1930) Dowson, 1939, ZI1–ZI3; *X. pelargonii* (Brown, 1923) Starr & Burkholder, 1942, ZJ1, ZJ2, ZJ5–ZJ9; *X. phaseoli* (Smith, 1897) Dowson, 1939, ZK1–ZK3; *X. phaseoli* var. *fuscans* (Burkholder, 1930) Starr & Burkholder, 1942, ZK4–ZK7; *X. phaseoli* var. *sojense* (Hedges, 1922) Starr & Burkholder, 1942, ZK8–ZK10; *X. physalidicola* Goto & Okabe, 1958, YZ1; *X. pisi* Goto & Okabe, 1958, XA1; *X. poinsettiaecola* Patel *et al.*, 1951, ZX1; *X. pruni* (Smith, 1903) Dowson, 1939, T1, T2, T6–T15; *X. punicae* Hingorani & Singh, 1959, YG1, YG2; *X. ricinicola* (Elliott, 1930) Dowson, 1939, ZM1–ZM4; *X. sesbaniae* Patel *et al.*, 1952, YH1, YH2; *X. tamarindi* Patel *et al.*, 1951, YY1; *X. taraxaci* Niederhauser, 1943, XF1; *X. translucens* (Jones *et al.*, 1917) Dowson, 1939, ZQ1–ZQ15; *X. trichodesmae* Patel *et al.*, 1952, YI1; *X. uppalii* Patel, 1948, ZR1–ZR3; *X. vasculorum* (Cobb, 1893) Dowson 1939, ZS1–ZS9; *X. vesicatoria* (Doidge, 1920) Dowson, 1939, X1, X3–X5, X7–X10, X12–X14; *X. vesicatoria* var. *raphani* (White, 1930) Starr & Burkholder 1942, X11; *X. vignicola* Burkholder, 1944, ZY1–ZY5; *X. vitians* (Brown, 1918) Dowson, 1943, YA1, YA3, YA4, *X. vitis-carnosae* Moniz & Patel, 1958, XH1.

For comparative purposes a number of non-xanthomonad cultures which produce yellowish growth on nutrient agar (Difco) were included in this study in the appropriate tests. An alphabetical listing of the codes used for the *Xanthomonas* cultures is given in Table 1.

EXPERIMENTAL METHODS

The media and methods used for examining the cultural, physiological and biochemical characters of the organisms were selected after extensive preliminary trials in which a number of media and methods were compared. Unless otherwise stated all incubation was at 27°C, including shaker incubated cultures. The shaker used had a horizontal reciprocating action of ⅞ in. at 280 rev/min.

Method of Inoculation

Cells from a young GYCA (glucose, 5 g; yeast extract (Difco), 5 g; calcium carbonate precip., 40 g; agar (Davis, N.Z.) 15 g; water 1 l) culture were dispersed in sterile water to give a faintly turbid suspension.

Using a 2 mm loop, one drop containing approximately 200–500 bacteria was spread over the surface of agar media slopes in test tubes. Liquid media were inoculated by the addition of two drops from a sterile Pasteur pipette.

TABLE 1—Alphabetical List of Codes Used to Designate the Cultures Tested in This Study

Code	Culture	Code	Culture
Q	= *X. campestris*	YP	= *X. barbarae*
R	= *X. citri*	YQ	= *X. corylina*
S	= *X. juglandis*	YR	= *X. cajani*
T	= *X. pruni*	YS	= *X. clerodendri*
X	= *X. vesicatoria*	YT	= *X. erythrinae*
Y	= *X. hyacinthi*	YV	= *X. hederae*
Z	= *X. begoniae*	YW	= *X. holcicola*
		YX	= *X. incanae*
		YY	= *X. tamarindi*
XA	= *X. pisi*	YZ	= *X. physalidicola*
XB	= *X. badrii*		
XD	= *X. dieffenbachiae*		
XE	= *X. desmodiigangeticii*	ZB	= *X. axonopodis*
XF	= *X. taraxaci*	ZC	= *X. carotae*
XG	= *X. martinicola*	ZD	= *X. cassava*
XH	= *X. vitis-carnosae*	ZE	= *X. cucurbitae*
		ZF	= *X. lactucae-scariolae*
		ZG	= *X. malvacearum*
YA	= *X. vitians*	ZH	= *X. manihotis*
YB	= *X. geranii*	ZI	= *X. papavericola*
YC	= *X. betlicola*	ZJ	= *X. pelargonii*
YD	= *X. cassiae*	ZK	= *X. phaseoli*
YE	= *X. desmodii*	ZM	= *X. ricinicola*
YF	= *X. nakatae*	ZQ	= *X. translucens*
YG	= *X. punicae*	ZR	= *X. uppalii*
YH	= *X. sesbaniae*	ZS	= *X. vasculorum*
YI	= *X. trichodesmae*	ZT	= *X. nigromaculans*
YK	= *X. oryzae*	ZU	= *X. alfalfae*
YL	= *X. lespedezae*	ZV	= *X. beticola*
YM	= *X. maculifoliigardeniae*	ZX	= *X. poinsettiaecola*
YN	= *X. lawsoniae*	ZY	= *X. vignicola*
YO	= *X. amaranthicola*		

Microscopical Characters

MORPHOLOGY AND GRAM REACTION

Air dried films of 24 h GYCA cultures were stained by the method of Gram as modified by Hucker (Society of American Bacteriologists 1957; p. 16).

MOTILITY AND FLAGELLA STAINING

Cultures were transferred twice at 24 h intervals to yeast extract—salts (YS) agar slopes, ($NH_4H_2PO_4$, 0·5 g; K_2HPO_4, 0·5 g; $MgSO_4 \cdot 7H_2O$, 0·2 g; NaCl, 5 g; yeast extract (Difco), 5 g; agar (Davis N.Z.), 12 g;

water 1 l) on which xanthomo
for motility. Non-motile cultur
and YS broth (as for YS aga
24, and 48 h. Flagella were sta

Gr

COLOUR AND SLIME FORMATIO

Four media were used for th
viz. GYCA, potato wedges, P
citrate, 1 g; peptone, 5 g; dextr
15 g; extract of 300 g cooked
PDA, 2 in which the Na_2HP
pH 7·3.

OXYGEN REQUIREMENTS

These were tested by deep
freshly prepared "glucose aga
tubes containing glucose 5 g;
8 g; brom cresol purple, 0·01
inoculation the agar surface in
a layer of sterile melted petrol
a depth of ½ to half inch.

THE EFFECT OF TEMPERATURE

The minimum temperature
slopes incubated in a stirred
of 5°C, 7°C, and 9°C, ± 0·5
for growth was observed on
incubated in stirred waterbaths
40°C ± 0·25°C for 12 days.

THE EFFECT OF NaCl CONCENT

Tolerance of sodium chlori
broth containing concentrations o
tubes of YS broth without add
examined for turbidity over a p
being reinoculated into the sam
check further the upper limit

Utilisation

MODE OF UTILISATION OF GLUC

This was determined by Hugh
used being trypticase (Baltim
digest of casein.

ıtaining approximately 200–500 bacteria
ıgar media slopes in test tubes. Liquid
tion of two drops from a sterile Pasteur

les Used to Designate the Cultures Tested
This Study

YP	=	*X. barbarae*
YQ	=	*X. corylina*
YR	=	*X. cajani*
YS	=	*X. clerodendri*
YT	=	*X. erythrinae*
YV	=	*X. hederae*
YW	=	*X. holcicola*
YX	=	*X. incanae*
YY	=	*X. tamarindi*
YZ	=	*X. physalidicola*
ZB	=	*X. axonopodis*
ZC	=	*X. carotae*
ZD	=	*X. cassava*
ZE	=	*X. cucurbitae*
ZF	=	*X. lactucae-scariolae*
ZG	=	*X. malvacearum*
ZH	=	*X. manihotis*
ZI	=	*X. papavericola*
ZJ	=	*X. pelargonii*
ZK	=	*X. phaseoli*
ZM	=	*X. ricinicola*
ZQ	=	*X. translucens*
ZR	=	*X. uppalii*
ZS	=	*X. vasculorum*
ZT	=	*X. nigromaculans*
ZU	=	*X. alfalfae*
ZV	=	*X. beticola*
ZX	=	*X. poinsettiaecola*
ZY	=	*X. vignicola*

ıpical Characters

ON

\ cultures were stained by the method of
ıociety of American Bacteriologists 1957;

NG

ice at 24 h intervals to yeast extract—
O_4, 0·5 g; K_2HPO_4, 0·5 g; $MgSO_4{\cdot}7H_2O$,
(Difco), 5 g; agar (Davis N.Z.), 12 g;

water 1 l) on which xanthomonads grow readily and were then examined for motility. Non-motile cultures were transferred to further YS agar slopes and YS broth (as for YS agar but without agar) and examined after 12, 24, and 48 h. Flagella were stained by Gray's (1926) method.

Growth Characters

COLOUR AND SLIME FORMATION

Four media were used for the observation of colour and slime formation, viz. GYCA, potato wedges, PDA, 1 (Na_2HPO_4, 2 g; NaCl, 2 g; sodium citrate, 1 g; peptone, 5 g; dextrose, 6 g; asparagine, 1 g; agar (Davis N.Z.), 15 g; extract of 300 g cooked potato; water to make up 1 l; pH 6·7) and PDA, 2 in which the Na_2HPO_4 in PDA, 1 was substituted by Na_3PO_4; pH 7·3.

OXYGEN REQUIREMENTS

These were tested by deep stab inoculations into duplicate tubes of freshly prepared "glucose agar" (10 ml quantities in 16 mm diam. test tubes containing glucose 5 g; peptone 5 g; Lab-lemco (Oxo), 5 g; agar 8 g; brom cresol purple, 0·014 g; water 1 l, pH 7·0). Immediately after inoculation the agar surface in one of each pair of tubes was covered with a layer of sterile melted petroleum jelly/liquid paraffin mixture (1 : 1) to a depth of ¼ to half inch.

THE EFFECT OF TEMPERATURE

The minimum temperature for growth was determined on YS agar slopes incubated in a stirred low temperature waterbath at temperatures of 5°C, 7°C, and 9°C, ± 0·5°C for 12 days. The maximum temperature for growth was observed on YS agar slopes and in YS broth cultures incubated in stirred waterbaths having temperatures ranging from 33°C to 40°C ± 0·25°C for 12 days.

THE EFFECT OF NaCl CONCENTRATION

Tolerance of sodium chloride was observed in shaker incubated YS broth containing concentrations of from 1 – 10% NaCl in 1% steps. Control tubes of YS broth without added NaCl were also inoculated. Tubes were examined for turbidity over a period of 10 days, cultures showing turbidity being reinoculated into the same NaCl concentration by serial transfer to check further the upper limit of growth.

Utilisation of Carbon Compounds

MODE OF UTILISATION OF GLUCOSE

This was determined by Hugh and Leifson's (1953) method, the peptone used being trypticase (Baltimore Biological Laboratories), a pancreatic digest of casein.

PRODUCTION OF ACID FROM CARBOHYDRATES AND RELATED CARBON SOURCES

This was observed from agar slopes of medium C. ($NH_4H_2PO_4$, 0·5 g; K_2HPO_4, 0·5 g; $MgSO_4{\cdot}7H_2O$, 0·2 g; NaCl, 5 g; yeast extract (Difco), 1 g; agar 12 g; water 1 l + brom cresol purple, 0·7 ml of 1·5% alcohol solution; pH 6·8, heat sterilised) + carbon source, 0·5% (v/v) added aseptically from Steiz filter-sterilised, concentrated solutions. The carbon sources tested were arabinose, rhamnose, xylose, glucose, fructose, galactose, mannose, ribose, lactose, sucrose, maltose, trehalose, melibiose, cellobiose, raffinose, melezitose, starch, inulin, dextrin, glycogen, glycerol, adonitol, mannitol, sorbitol, dulcitol, inositol, salicin, and α-methyl-D-glucoside. Because of the difficulty of dissolving and filter sterlising even 5% (w/v) solutions of starch, inulin, glycogen and salicin, these carbon sources were, for comparison, also prepared by dissolving in a larger volume of water added to medium C and sterilising in steam on 3 successive days. Cultures were examined for growth, and acid or gas production after 2, 4 and 7 days, then at 7-day intervals to 42 days.

METHYL RED TEST

To examine methyl red as an indicator of the final pH during glucose utilisation, the medium found most sensitive contained $NH_4H_2PO_4$, 0·5 g; K_2HPO_4, 0·5 g; $MgSO_4{\cdot}7H_2O$, 0·2 g; NaCl 5 g; yeast extract, 1 g; glucose, 10 g; water, 1 l; pH 6·8.

UTILISATION OF ORGANIC ACIDS

The medium used consisted of YS agar with the yeast extract reduced to 0·08% (w/v) + salt of the organic acid, 0·2% (w/v) + brom thymol blue 0·0016% (w/v) (water solution); pH adjusted with NaOH to 6·8 before autoclaving. The organic acids used were acetic, benzoic, citric, lactic, malic, oxalic, propionic, succinic and tartaric as sodium salts and gluconic as the calcium salt.

UTILISATION OF ASPARAGINE AS THE SOLE SOURCE OF CARBON AND NITROGEN

Four inorganic solutions were prepared viz. 1 = K_2HPO_4, 8 g; KH_2PO_4, 2 g; distilled water, 100 ml. 2 = $MgSO_4{\cdot}7H_2O$, 2 g; $FeSO_4$, 0·5 g; NaCl, 1 g; $MnSO_4$, 0·02 g; 1 drop N_2-free H_2SO_4; dist. water, 100 ml. 3 = Na_2MoO_4, 0·02 g; dist. water, 100 ml. 4 = $CaSO_4$, saturated solution in dist. water. 10 ml of each solution were mixed in the order 3, 4, 2, 1, filtered and added to 960 ml dist. water in which was dissolved 2 g l-asparagine. This was then dispensed in 5 ml quantities in test tubes. All glassware used was acid soaked then thoroughly rinsed in 4 changes of distilled water and dried before use. Tubes were examined for growth after 4, 7, and 10 days shaker incubation. Weak growth was checked by transferring twice a 2 mm loopful of the 10-day-old culture to fresh tubes of the asparagine medium and to YS broth.

CATALASE PRODUCTION

A loopful of solid gr
into a drop of 10 vol. h
examined for the produ

GELATIN HYDROLYSIS

Stab inoculations we
at 22°C and recorded a
mate amount of liquefa
tures were also examin
described by Smith *et al*
around colonies was me

ACTION IN MILK

This was observed
milk + brom cresol pu
cessive days. Results we
from that of Smith *et*
were prepared; over th
nutrient agar (YNA)
which 15% (v/v) steril
dried for 2 h at 45°C
and incubated for 6 days

INDOLE PRODUCTION

Cultures were inocula
10 g; yeast extract, 5 g
2 and 4 days by the va

ACETOIN PRODUCTION

Cultures were grown
tested for the presence o
to 1 ml of culture was
alcohol and 0·2 ml of
shaken and the final r

UREASE PRODUCTION

A modified YS brot
cresol red 0·0016%) wa
urea solution was then a
was dispensed aseptically
presence of yeast extrac
sets of check tubes, i.e.
urea inoculated. A mark
the presence of urease.

ʌTES AND RELATED CARBON

'um C. ($NH_4H_2PO_4$, 0·5 g; yeast extract (Difco), 1 g; nl of 1·5% alcohol solu- ', (v/v) added aseptically ıe carbon sources tested galactose, mannose, cellobiose, raffinose, adonitol, mannitol, .ide. Because of the .% (w/v) solutions of ..bon sources were, for com- a larger volume of water added ..n on 3 successive days. Cultures were or gas production after 2, 4 and 7 days, ys.

indicator of the final pH during glucose ıost sensitive contained $NH_4H_2PO_4$, 0·5 g; ı·2 g; NaCl 5 g; yeast extract, 1 g; glucose,

of YS agar with the yeast extract reduced organic acid, 0·2% (w/v) + brom thymol ılution); pH adjusted with NaOH to 6·8 acids used were acetic, benzoic, citric, lactic, : and tartaric as sodium salts and gluconic

AS THE SOLE SOURCE OF CARBON AND

prepared viz. 1 = K_2HPO_4, 8 g; KH_2PO_4, = $MgSO_4 \cdot 7H_2O$, 2 g; $FeSO_4$, 0·5 g; NaCl, ? N_2-free H_2SO_4; dist. water, 100 ml. ıter, 100 ml. 4 = $CaSO_4$, saturated solution ılution were mixed in the order 3, 4, 2, 1, t. water in which was dissolved 2 g l-aspara- ı 5 ml quantities in test tubes. All glassware ·ughly rinsed in 4 changes of distilled water vere examined for growth after 4, 7, and k growth was checked by transferring twice ıld culture to fresh tubes of the asparagine

Biochemical Tests

CATALASE PRODUCTION

A loopful of solid growth taken from a 24 h GYCA slope was removed into a drop of 10 vol. hydrogen peroxide solution on a clear glass plate and examined for the production of gas bubbles.

GELATIN HYDROLYSIS

Stab inoculations were made into nutrient gelatin (Difco), incubated at 22°C and recorded after 4, 7, 21, and 28 days, the shape and approximate amount of liquefaction when present being noted. Some of the cultures were also examined by the modification of Frazier's (1926) method described by Smith *et al.* (1952), in which instances the zone of hydrolysis around colonies was measured after 2 and 6 days.

ACTION IN MILK

This was observed in "purple milk" (reconstituted powdered skim milk + brom cresol purple, 0·04 g/l) sterilised by steaming on three successive days. Results were confirmed on milk agar plates, a method modified from that of Smith *et al.* (1952)—viz. thin nutrient agar (Difco) plates were prepared; over this agar surface was poured a layer of yeast extract nutrient agar (YNA) (nutrient agar + 0·5% (w/v) yeast extract) to which 15% (v/v) sterile skim milk had been added aseptically. Plates were dried for 2 h at 45°C then spot inoculated with four cultures per plate and incubated for 6 days.

INDOLE PRODUCTION

Cultures were inoculated into a medium consisting of tryptone (Difco), 10 g; yeast extract, 5 g; water 1 l, incubated on a shaker and tested after 2 and 4 days by the vanillin method (Roessler and McClung 1943).

ACETOIN PRODUCTION

Cultures were grown in YS broth + glucose 5 g/l, shaker incubated and tested for the presence of acetylmethylcarbinol (acetoin) after 2 and 5 days; to 1 ml of culture was added 0·6 ml of 5% (w/v) α-naphthol in absolute alcohol and 0·2 ml of 40% (w/v) KOH. The tubes were vigorously shaken and the final reading was taken after 2 hr.

UREASE PRODUCTION

A modified YS broth (yeast extract reduced to 0·1% and containing cresol red 0·0016%) was prepared and autoclaved in flasks. Filter sterilised urea solution was then added to make a 2% concentration and the medium was dispensed aseptically in 5 ml quantities in sterile test tubes. Due to the presence of yeast extract in the medium it was necessary to have double sets of check tubes, i.e., (a) with urea, not inoculated, and (b) without urea inoculated. A marked increase in alkalinity was regarded as showing the presence of urease.

PRODUCTION OF NITRITE FROM NITRATE

The medium used consisted of: KH_2PO_4, 0·5 g; K_2HPO_4, 0·5 g; $MgSO_4 \cdot 7H_2O$, 0·2 g; NaCl, 5 g; yeast extract, 5 g; sodium succinate, 2 g; potassium nitrate, 1 g; distilled water, 1 l. Two other media, viz., nitrate peptone water (potassium nitrate, 1 g; peptone, 10 g; water 1 l) and synthetic nitrate medium (Society of American Bacteriologists 1942) K_2HPO_4, 0·5 g; $CaCl_2$ (anhyd.), 0·5 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; glucose, 10 g; KNO_3, 1 g; distilled water 1 l) proved unsatisfactory for use with xanthomonads largely because some organisms failed to grow in them. Cultures were shaker incubated and tested for nitrite with sulphanilic acid and dimethyl-α-naphthylamine, after 1, 2, and 4 days. Zinc dust was used to ensure that negative reactions were not due to reduction of nitrate beyond the nitrite stage.

AMMONIA PRODUCTION

Cultures were grown in a medium containing meat extract (Oxo Lab-lemco), 3 g; peptone (Difco), 5 g; yeast extract (Difco), 1 g; water 1 l. Results were recorded after after 2 and 4 days' shaker incubation; 3 drops (Pasteur pipette) of culture were mixed with 3 drops of Nessler's solution on a white spot tile and the resultant colour was compared with those produced by 0·1% and 0·05% (w/v) solutions of $NH_4H_2PO_4$.

HYDROGEN SULPHIDE PRODUCTION

Three media were selected to demonstrate the production of hydrogen sulphide, viz. (1) YS broth + cysteine hydrochloride 0·1 g/l. (2) YS broth + sodium thiosulphate 0·5 g/l (a concentration of 0·2% (w/v) inhibited many organisms). (3) YS broth + peptone, 0·5 g/l. Cultures were shaker incubated and results recorded after 3, 6, and 14 days, the presence of H_2S being indicated by lead acetate strips suspended over the medium.

HYDROLYSIS OF STARCH

Two media were used, viz. (1) yeast extract nutrient agar (YNA) (yeast extract, 5 g; peptone, 5 g; Lab-lemco (Oxo), 5 g; water 1 l; pH 6.8) + potato starch (Difco) 1% (w/v). (2) YNA + soluble starch (Difco) 0·2% (w/v). Plates were dried for 2 h at 45°C, then spot inoculated in duplicate. After 2–4 days depending on growth, one colony of each culture was wiped from the agar surface before flooding with dilute iodine solution.

HYDROLYSIS OF AESCULIN

This was observed in YS broth + aesculin, 1 g; ferric ammonium citrate, 0·5 g/l; pH 6·8. Results were recorded in daylight and U.V. light.

HYDROLYSIS OF SODIUM HIPPURATE

Two media were used; (1) YS broth + sodium hippurate, 10 g/l. (2) YS broth + sodium hippurate, 10 g; glucose 5 g; peptone, 1 g/l. Cultures were tested after 5 and 10 days for white benzoic acid crystals by

two methods. (a) To 1 ml
reagent (ferric chloride, 12%
in tap water) was added. This
slowly to check tubes sufficien
occurred when the first 0·1 ml
1 ml 2N sulphuric acid was ad
allowed to stand and were rec

LIPOLYTIC ACTIVITY

The fat emulsion agar pla
test (Paton, 1956) were used

TYROSINASE ACTIVITY

Cultures were grown (shake
L-tyrosine (Difco) (Burkholde

ACTION ON URIC ACID

This was tested by the me
nutrient agar was poured in P
YNA containing 0·2% (w/v)
for 2 h at 45°C then spot inocu

ACTION ON NUCLEIC ACID

The method of Jeffries, Hol

ACTION ON EGG YOLK

A method modified from
used; viz., yolk from fresh he
quantity of sterile sodium chlo
shaking, 5 ml of egg yolk sali
at 47°C and plates were pour
5% (w/v) egg yolk saline to

PECTOLYTIC ACTIVITY

The pectolytic activity of m
has already been published (D

RESULTS AN

Micro

MORPHOLOGY AND GRAM REA

All cultures proved to be Gra

MOTILITY AND FLAGELLATION

All cultures examined prov
trichous polar flagella.

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f: KH_2PO_4, 0·5 g; K_2HPO_4, 0·5 g;
east extract, 5 g; sodium succinate, 2 g;
ter, 1 l. Two other media, viz., nitrate
g; peptone, 10 g; water 1 l) and syn-
merican Bacteriologists 1942) K_2HPO_4,
$O_4 \cdot 7H_2O$, 0.2 g; glucose, 10 g; KNO_3,
satisfactory for use with xanthomonads
ailed to grow in them. Cultures were
rite with sulphanilic acid and dimethyl-
days. Zinc dust was used to ensure that
reduction of nitrate beyond the nitrite

um containing meat extract (Oxo Lab-
; yeast extract (Difco), 1 g; water 1 l.
and 4 days' shaker incubation; 3 drops
nixed with 3 drops of Nessler's solution
nt colour was compared with those pro-
solutions of $NH_4H_2PO_4$.

emonstrate the production of hydrogen
cysteine hydrochloride 0·1 g/l. (2) YS
g/l (a concentration of 0·2% (w/v)
YS broth + peptone, 0·5 g/l. Cultures
recorded after 3, 6, and 14 days, the
y lead acetate strips suspended over the

) yeast extract nutrient agar (YNA)
; Lab-lemco (Oxo), 5 g; water 1 l;
1% (w/v). (2) YNA + soluble starch
dried for 2 h at 45°C, then spot inocu-
s depending on growth, one colony of
agar surface before flooding with dilute

th + aesculin, 1 g; ferric ammonium
ere recorded in daylight and U.V. light.

E

YS broth + sodium hippurate, 10 g/l.
ate, 10 g; glucose 5 g; peptone, 1 g/l.
0 days for white benzoic acid crystals by

two methods. (a) To 1 ml portions of culture, 0·3 ml ferric chloride reagent (ferric chloride, 12% (w/v); conc. hydrochloric acid, 0·25% (v/v) in tap water) was added. This amount of reagent was determined by adding slowly to check tubes sufficient reagent to redissolve the precipitate which occurred when the first 0·1 ml was added. (b) To 1 ml portions of culture 1 ml 2N sulphuric acid was added. The tubes were shaken occasionally then allowed to stand and were recorded after 30 to 60 minutes.

LIPOLYTIC ACTIVITY

The fat emulsion agar plate method of Paton (1956) and the rapid test (Paton, 1956) were used.

TYROSINASE ACTIVITY

Cultures were grown (shaker incubated) in YS broth + 0·05% (w/v) L-tyrosine (Difco) (Burkholder and Starr, 1948) and recorded after 4 days.

ACTION ON URIC ACID

This was tested by the method of Schefferle (1957) (i.e., a layer of nutrient agar was poured in Petri dishes; over this was poured a layer of YNA containing 0·2% (w/v) uric acid suspension; the plates were dried for 2 h at 45°C then spot inoculated with 4 cultures per plate.

ACTION ON NUCLEIC ACID

The method of Jeffries, Holtman and Guse (1957) was used.

ACTION ON EGG YOLK

A method modified from that of McGaughey and Chu (1948) was used; viz., yolk from fresh hen's eggs was mixed aseptically with an equal quantity of sterile sodium chloride solution (0·85% w/v); after thoroughly shaking, 5 ml of egg yolk saline was transferred to 100 ml of liquid YNA at 47°C and plates were poured. Egg yolk broth was prepared by adding 5% (w/v) egg yolk saline to 5 ml quantities of YS broth.

PECTOLYTIC ACTIVITY

The pectolytic activity of many of the organisms included in this study has already been published (Dye 1960).

RESULTS AND DISCUSSION OF RESULTS

Microscopical Characters

MORPHOLOGY AND GRAM REACTION

All cultures proved to be Gram-negative rods.

MOTILITY AND FLAGELLATION

All cultures examined proved to be actively motile and to have monotrichous polar flagella.

Growth Characters

COLOUR AND SLIME FORMATION

Most of the cultures produced a yellowish growth on the several media tested but ZH1–ZH3, (*X. manihotis*) produced white growth. The yellow pigment was shown to be soluble in alcohol but not in water. The yellow colours produced were identified according to Ridgway's (1912) charts. There was considerable variation in the colours produced by cultures of one species on one or several media, by cultures when repeated on the same medium and by the same culture on two slightly different media (e.g., on PDA, 1 and PDA, 2). The colours were also found to change with age of the culture. It is not surprising therefore that the colours observed here were different from those recorded by other workers.

A number of cultures were found to produce a diffusable brown pigmentation of liquid and solid media such as GYCA, YSA, YNA, YS broth, YN broth. These cultures were Q21, *X. campestris*; YG1, YG2, *X. punicae*; ZK4, ZK6, ZK7, *X. phaseoli* var. *fuscans*; ZM2, ZM3, *X. ricinicola*. Unexpectedly, ZK5, ZM1, and ZM4 did not produce a brown colour in these media though ZK5 did so in a tyrosine medium (see later section) along with a number of other cultures. Since production of the brown pigment is not rare in the genus and may not even be a stable character there seems little justification for separating *X. phaseoli* var. *fuscans* from *X. phaseoli*.

Most xanthomonads produced a copious slime when grown on agar media containing glucose or a number of other carbohydrates but some xanthomonads including *X. hyacinthi* exhibited a tendency to produce little and sometimes no obvious slime after being held on artificial media for more than 12 months. By contrast an increase in the degree of mucoidness with serial transfer on media rich in carbohydrate has been noted by Elrod and Braun (1947) and Hayward and Hodgkiss (1961).

Experience shows that slime production, and the ability to produce a non-diffusible yellow pigment or a diffusable brown pigment, are unreliable diagnostic characters in the genus *Xanthomonas* though slime formation has some value in screening cultures.

OXYGEN REQUIREMENTS

All cultures grew on the surface only of the open tubes and if any change occurred in pH this was confined to the top 10 mm. No obvious growth and no pH change occurred beneath the oil layer. Therefore all cultures were considered to be strict aerobes.

THE EFFECT OF TEMPERATURE

MINIMUM No xanthomonad was able to grow at 5°C though most grew at 7°C; some were however unable to grow at 9°C. Species differentiation was not possible using this test.

MAXIMUM It was found that many cultures grew at temperatures 2° higher in broth than on agar, which was contrary to expectation. The reason

for this was not determined. No
YS broth. Some could grow at 39
at 33°C. The temperature maxim

THE EFFECT OF NaCl CONCENT

The maximum concentraton o
frequently reported (Bergey 195
Starr (1948) salt tolerance has re
and frequently the published res
prohibited comparison with those
of a comparative study and indic
cultures of the same species. Gro
lower than the figure quoted. X
salt tolerant since no culture was
and very few could tolerate 5%
species could not be differentiated

TABLE 2— Maximum Concentrati
in

Culture
ZB1* ....
YK1, YK2, YV2, YV3, ZD1, ZG2, Z
R1, R5, R14, R15, S1, S2, S10–S15 X9, X10, X12, X13, Y2, Y7, Z3, X YA4, YG1, YG2, YK3–YK5, YL YT2, YV1, YX1, YZ1, ZE1, ZF1, ZI1, ZJ5, ZJ8, ZJ9, ZK1–ZK5, ZK ZQ7, ZQ13–ZQ15, ZS1–ZS3, ZS5,
Q2, Q6–Q8, Q11–Q17, Q20–Q25, S5–S9, T1–T9, T11, T13, T15, X7, Z2, XA1, XB1, XE1, XH1, YB1, Y YF2, YM2, YN1, YO1, YP2, YP4 ZC1–ZC4, ZE2, ZG3, ZG4, ZG11– ZK6, ZK8–ZK10, ZQ3, ZQ5, ZQ6 ZR3, ZS4, ZS9, ZT1, ZV3, ZX1, Z
Q5, Q18, Q19, R12, R13, S4, YA1, Y YM1, ZI2, ZI3, ZM2–ZM4, ZU1
Many yellow pigmented non-xanthomon

*Yeast extract 0·5% + NaCl.

th Characters

a yellowish growth on the several media
otis) produced white growth. The yellow
: in alcohol but not in water. The yellow
according to Ridgway's (1912) charts.
in the colours produced by cultures of
nedia, by cultures when repeated on the
culture on two slightly different media
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var. *fuscans*; ZM2, ZM3, *X. ricinicola*.
ZM4 did not produce a brown colour in
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able to grow at 9°c. Species differentiation

at many cultures grew at temperatures 2°
hich was contrary to expectation. The reason

for this was not determined. No xanthomonad was able to grow at 40°c in YS broth. Some could grow at 39°c and others at 35°c. All showed growth at 33°c. The temperature maxima did not differentiate species.

THE EFFECT OF NaCl CONCENTRATION

The maximum concentraton of sodium chloride permitting growth is frequently reported (Bergey 1957) but as pointed out by Burkholder and Starr (1948) salt tolerance has received comparatively little systematic study and frequently the published results were obtained under conditions that prohibited comparison with those of other workers. Table 2 presents results of a comparative study and indicates that there is much variation between cultures of the same species. Growth was considerably better at 1% units lower than the figure quoted. *Xanthomonas* as a group is not particularly salt tolerant since no culture was able to grow in the presence of 6% NaCl and very few could tolerate 5%. It is considered that the *Xanthomonas* species could not be differentiated by this test.

TABLE 2— Maximum Concentration of Sodium Chloride Permitting Growth in YS Broth

Culture	Concentration
ZB1*	1%
YK1, YK2, YV2, YV3, ZD1, ZG2, ZQ11, ZR1, ZS7	2%
R1, R5, R14, R15, S1, S2, S10–S15, T10, T12, T14, X1–X5, X9, X10, X12, X13, Y2, Y7, Z3, XD1, XE2, XF1, XG1, YA3, YA4, YG1, YG2, YK3–YK5, YL1, YQ1, YQ2, YR1, YT1, YT2, YV1, YX1, YZ1, ZE1, ZF1, ZG1, ZG5–ZG7, ZH1–ZH3, ZI1, ZJ5, ZJ8, ZJ9, ZK1–ZK5, ZK7, ZM1, ZQ1, ZQ2, ZQ4, ZQ7, ZQ13–ZQ15, ZS1–ZS3, ZS5, ZS6, ZS8, ZT2, ZY3–ZY5	3%
Q2, Q6–Q8, Q11–Q17, Q20–Q25, R2–R4, R6, R8–R11, S3, S5–S9, T1–T9, T11, T13, T15, X7, X8, X11, X14, Y5, Y6, Z1, Z2, XA1, XB1, XE1, XH1, YB1, YB2, YD1, YD2, YE2, YF1, YF2, YM2, YN1, YO1, YP2, YP4, YS1, YW1, YX2, YY1, ZC1–ZC4, ZE2, ZG3, ZG4, ZG11–ZG13, ZJ1, ZJ2, ZJ6, ZJ7, ZK6, ZK8–ZK10, ZQ3, ZQ5, ZQ6, ZQ8–ZQ10, ZQ12, ZR2, ZR3, ZS4, ZS9, ZT1, ZV3, ZX1, ZY1, ZY2	4%
Q5, Q18, Q19, R12, R13, S4, YA1, YC1, YE1, YH1, YH2, YI1, YM1, ZI2, ZI3, ZM2–ZM4, ZU1	5%
Many yellow pigmented non-xanthomonads	6–10% or more

*Yeast extract 0·5% + NaCl.

Utilisation of Carbon Compounds

MODE OF UTILISATION OF GLUCOSE

All cultures exhibited a strictly oxidative metabolism of glucose and it is considered that this feature should be included in the definition of the genus.

PRODUCTION OF ACID FROM CARBOHYDRATES AND RELATED CARBON SOURCES

The results are summarised in Table 3. The medium used was particularly sensitive to acid production; any marked increase in its content of yeast extract prevented the demonstration of acid formation by xanthomonads. It appears therefore that these organisms do not accumulate more than minute traces of acids. Delayed acid production was in some instances due to mutation as was indicated by the appearance after a few days of some large acid-forming colonies. Other cases of delayed acid production could not be so explained. There were some cases of a carbon source apparently being utilised, as indicated by increased growth and slime formation, without apparent acid production, e.g., cultures XA1 (*X. pisi*) and XB1, (*X. badrii*) on salicin and XB1 on α-methyl-D-glucoside; many cultures appeared able to utilise glycogen without evidence of acid production.

It is considered that little significance can be placed on the ability of *Xanthomonas* to produce acid from carbohydrates and related carbon sources and that the species can not be unequivocally identified by their acid production from or utilisation of any or all of the carbon sources tested here.

Possibly some use could be made of tests for acid formation for screening xanthomonads from non-xanthomonads if the basal medium employed here is used with the addition of rhamnose, inulin, adonitol, sorbitol, dulcitol, or inositol. Of these materials, rhamnose and inositol appear to be the most suitable since they are readily utilised by many non xanthomonads; inulin, adonitol, sorbitol, and dulcitol are less frequently utilised.

METHYL RED TEST

Very few cultures were able to lower the pH value to 4·6 or below in the medium used though most lowered it to 5·0–5·2. There was a lack of consistency in the results given by different cultures of the same species and in repeated trials with the same cultures. This test was therefore considered to have no value for distinguishing *Xanthomonas* species.

UTILISATION OF ORGANIC ACIDS

All the cultures with the exception of ZB1 (*X. axonopodis*), gave the following results:

(a) They utilised acetate, citrate, malate, propionate, and succinate; with the exception of Y2–Y7 (*X. hyacinthi*) and YG1, YG2 (*X. punicae*) all utilised lactate:

(b) They were unable to u
utilise gluconate:

(c) They were inhibited by
to grow weakly after 10–14 d
podis) did not grow on any

TABLE 3—Production of Acid
21 D

	Posit
Arabinose	56
Rhamnose	
Xylose	47
Glucose	57
Fructose	56
Galactose	56
Mannose	56
Ribose	16
Lactose	26
Sucrose	55
Maltose	36
Trehalose	48
Melibiose	44
Cellobiose	56
Raffinose	20
Melezitose	
Inulin	
Dextrin	36
Adonitol	
Mannitol	12
Sorbitol	
Dulcitol	
Inositol	
Salicin	
α-methyl-D-glucoside	
Glycogen‡	[illegible]

* = Negative within 21 days
negative result.
† = Variable either between s
between repeated tests of c
‡ = Only 36 cultures tested.
(a) = 8 gave a positive result onl
(b) = 2 cultures showed utilisatio
(c) = 21 cultures showed utilisat

Since there were no distinct
noted above, utilisation of th
Xanthomonas species. The acti
(1956) using manometric met
utilise gluconate. The above tes
some xanthomonads exhibit a d
was considered to be due eith
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f Carbon Compounds

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CARBOHYDRATES AND RELATED CARBON

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e, malate, propionate, and succinate; with
cinthi) and YG1, YG2 (*X. punicae*) all

(b) They were unable to utilise oxalate or tartrate and initially could not utilise gluconate:

(c) They were inhibited by benzoate; a few cultures were, however, able to grow weakly after 10–14 days on this medium. Culture ZB1 (*X. axonopodis*) did not grow on any of these media.

TABLE 3—Production of Acid from Carbohydrates and Related Sources within 21 Days from 57 Species

	Positive	Negative	Delayed+*	Variable†
Arabinose	56	1(ZB1)	...	...
Rhamnose	...	57	...	...
Xylose	47	4	3	3
Glucose	57	...	...	...
Fructose	56	...	1(ZB1)	...
Galactose	56	...	1(ZB1)	...
Mannose	56	1(ZB1)	...	...
Ribose	16	15	22	4
Lactose	26(a)	16	7	8
Sucrose	55	1	...	1
Maltose	36	16	...	5
Trehalose	48	1	...	8
Melibiose	44	8	...	5
Cellobiose	56	1(ZB1)	...	...
Raffinose	20	22	1	14
Melezitose	...	39	16	2
Inulin	...	57	...	...
Dextrin	36	15	...	6
Adonitol	...	57	...	...
Mannitol	12	35	2	8
Sorbitol	...	57	...	...
Dulcitol	...	57	...	...
Inositol	...	57	...	...
Salicin	...	57(b)	...	...
α-methyl-D-glucoside	...	56	...	1(XB1)
Glycogen‡	4	32(c)	...	...

* = Negative within 21 days but positive within 28–42 days; considered as a negative result.
† = Variable either between several cultures of a species at any one time or between repeated tests of one culture.
‡ = Only 36 cultures tested.
(a) = 8 gave a positive result only after apparent mutation.
(b) = 2 cultures showed utilisation without obvious acid production.
(c) = 21 cultures showed utilisation without obvious acid production.

Since there were no distinctions between species other than the three noted above, utilisation of the acids showed no value for identifying *Xanthomonas* species. The action on gluconate is of interest. Katznelson (1956) using manometric methods reported that xanthomonads do not utilise gluconate. The above tests confirm this in general but showed that some xanthomonads exhibit a delayed utilisation of gluconate. This ability was considered to be due either to adaptation by growing cells or to mutation.

UTILISATION OF ASPARAGINE AS THE SOLE SOURCE OF CARBON AND NITROGEN

Starr (1946) showed that asparagine is inadequate as a sole source of carbon and nitrogen for xanthomonads, and Burkholder has included this property in a definition of the genus (Bergey 1948 and 1957). The tests reported herein confirmed these findings and also showed that many yellow pigmented non-xanthomonads which were included for comparison were able to grow vigorously under these conditions.

Biochemical Tests

CATALASE PRODUCTION

All cultures gave a positive reaction.

GELATIN HYDROLYSIS

Most cultures liquefied gelatin at a moderate rate though some exhibited such slow weak liquefaction that if cultures had been recorded only in the first 7 days or if they had been incubated at 27°C, negative reactions would have been recorded; in these instances the liquid frequently evaporated as soon as it was formed. These cultures were YB1 (*X. geranii*), YK1–YK5 (*X. oryzae*), ZC3 (*X. carotae*), ZS1–ZS3, ZS5–ZS7 (*X. vasculorum*), ZY1–ZY3 (*X. vignicola*). The rate of liquefaction could not be used to define any species due to strain variation. One culture only (ZB1, *X. axonopodis*) was completely unable to liquefy gelatin within 28 days. This was confirmed in gelatin plates in which hydrolysis was not demonstrated by *X. axonopodis* (ZB1), or by 2/5 *X. oryzae* (YK3, YK5) and 5/9 *X. vasculorum* (ZS2, ZS3, ZS5–ZS7). The fact that *X. axonopodis* consistently did not exhibit gelatin hydrolysis is not necessarily valid for taxonomic purposes since only one culture was tested but it does confirm the result of Starr and Garces (1950). These tests demonstrate the unsuitability of gelatin hydrolysis as a diagnostic property of the genus *Xanthomonas* or of its species.

ACTION IN MILK

Proteolysis which was confirmed in milk agar plates was an outstanding characteristic of the group though some cultures gave a slow weak reaction. Only *X. axonopodis* was completely unable to clear the milk and this culture has already been noted as atypical in other respects. No culture produced an acid reaction in milk and this feature could possibly be used in the definition of the genus. Certainly it would be a useful characteristic in screening unknown xanthomonad-like organisms. Differentiation of the *Xanthomonas* species by their action on purple milk is considered impossible.

INDOLE PRODUCTION

The growth of most organisms was good, exceptions being YK1–YK5 (*X. oryzae*) and ZB1 (*X. axonopodis*) which grew slowly. Indole was not produced by xanthomonad cultures though it was produced by some non-

xanthomonads having a fermen
cluded for comparative purposes
"indole production by nonfer
(Hugh and Leifson 1953). Sk
monads produced indole but di
result it is considered that xan
the 8 cultures referred to by S

ACETOIN PRODUCTION

All xanthomonad cultures sho
It is suggested that this feature

UREASE PRODUCTION

No xanthomonads were able
monads tested at the same time

PRODUCTION OF NITRITE FROM

In preliminary tests it was foun
for several reasons and this ha
Starr (1948). The nitrate mediu
of the several that were examine
ZB1 (*X. axonopodis*) which gre
and 4 days (6–8 days for ZB1)

Xanthomonas ...
many non-*Xanthomonas*

Skerman (1959) reported that
nitrite. Other workers have also
Smith *et al.* (1911), *X. beticola*;
vericola; Takimoto (1934), *X. be*
Thornberry and Anderson (1937
Hipolito (1941), *X. manihotis*; D
holder and Starr (1948) reported
for the 2 isolates of *X. barbarae* f
single cultures of *X. cucurbitae* an
vericola and for about half of the

Cultures of all these and other
above medium and in additiona
incubation (the unshaken tubes be
instance was even a trace of nitrite
that xanthomonads do not accumu
tions reported herein.

AMMONIA PRODUCTION

The literature contains many ref
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tures though it was produced by some non-

xanthomonads having a fermentative metabolism of glucose, that were included for comparative purposes. This finding agrees with the statement that "indole production by nonfermenters has never been observed by us" (Hugh and Leifson 1953). Skerman (1959) reported that 8/46 xanthomonads produced indole but did not state the species. In view of the above result it is considered that xanthomonads do not produce indole and that the 8 cultures referred to by Skerman were not xanthomonads.

ACETOIN PRODUCTION

All xanthomonad cultures showed no production of acetyl-methyl-carbinol. It is suggested that this feature could be used in defining the genus.

UREASE PRODUCTION

No xanthomonads were able to produce urease whereas some pseudomonads tested at the same time gave strongly positive results.

PRODUCTION OF NITRITE FROM NITRATE

In preliminary tests it was found that reactions differed in different media for several reasons and this has also been reported by Burkholder and Starr (1948). The nitrate medium used in these tests was the most sensitive of the several that were examined. Growth was good in all cultures except ZB1 (*X. axonopodis*) which grew slowly. Results were recorded after 1, 2, and 4 days (6–8 days for ZB1) and can be summarised as follows:

Xanthomonas	Nitrite negative
many non-*Xanthomonas*	,, positive

Skerman (1959) reported that 14/59 xanthomonads reduce nitrate to nitrite. Other workers have also claimed positive reactions as follows: Smith *et al.* (1911), *X. beticola*; Bryan and McWhorter (1930), *X. papavericola*; Takimoto (1934), *X. begoniae*; Riker *et al.* (1935), *X. alfalfae*; Thornberry and Anderson (1937), *X. lactucae-scariolae*; Drummond and Hipolito (1941), *X. manihotis*; Dowson (1957), *X. ricinicola*. Also Burkholder and Starr (1948) reported "nitrate-reduction to nitrite was observed for the 2 isolates of *X. barbarae* for 2 of the 4 tested *X. begoniae*, for the single cultures of *X. cucurbitae* and *X. lactucae-scariolae*, for the 2 *X. papavericola* and for about half of the *X. geranii* and *X. pelargonii* isolates."

Cultures of all these and other xanthomonads were further tested in the above medium and in additional media, shaken and unshaken during incubation (the unshaken tubes being sealed with rubber bungs) but in no instance was even a trace of nitrite detected in 7 days. The writer believes that xanthomonads do not accumulate nitrite from nitrate under the conditions reported herein.

AMMONIA PRODUCTION

The literature contains many references (e.g., Burkholder, 1937; Dowson, 1957; Patel *et al.*, 1951, 1952, 1953, 1955; Sabet, 1959) stating that xanthomonads can "produce ammonia".

Using peptone as the source, this was confirmed in these tests since most cultures gave a Nessler colour reaction in 2–4 days equivalent to that given by 0·1% (w/v) ammonium dihydrogen phosphate. No species differentiation was possible using this test.

Hydrogen Sulphide Production

Preliminary tests showed that broth media if shaken during incubation gave a strong reaction sooner than agar slopes of similar media. Unshaken broth media gave a weak reaction slowly presumably due to slow growth of the organisms and to hydrogen sulphide remaining in solution. Preliminary tests also showed that although lead acetate strips were more sensitive than other methods tested for indicating the presence of H_2S, strips showing weakly positive results in 3–6 days sometimes exhibited in 6–14 days a change of the black lead sulphide to a white or colourless substance, possibly lead sulphate or lead carbonate and therefore appeared negative at that time. It was for these reasons that cultures were grown in broth media, shaken during incubation, and recorded after 3, 6, and 14 days.

All the xanthomonads utilised cysteine with the production of H_2S and all except YG2 (*X. punicae*) produced H_2S from peptone; most cultures produced H_2S from thiosulphate the exceptions being 1/3 *X. begoniae*, 1/2 *X. geranii*, 1/10 *X. malvacearum*, 2/2 *X. punicae*, 1/1 *X. trichodesmae*, 3/9 *X. vasculorum*, 1/5 *X. vignicola*. These results do not agree completely with those of other workers as shown in Table 4. This merely serves to emphasise the fact that this test cannot be used for the differentiation of *Xanthomonas* species.

TABLE 4—A Comparison of H_2S Production Determined by Various Workers

Species	Result	Reference	Result Here*
X. juglandis	Negative	Dowson (1957)	Positive 1, 2, & 3
X. maculifoliigardeniae	Negative	Ark (1946)	Positive 1, 2, & 3
X. nakatae	Type A negative Type B slight	Bergey (1957)	Positive 1, 2, & 3
X. vasculorum	Negative	Dowson (1957)	Positive 1 & 3 Variable 2
X. vitians	Feeble	Bergey (1957)	Positive 1, 2, & 3

*1 = cysteine medium
2 = sodium thiosulphate medium
3 = peptone medium

Hydrolysis of Starch

The xanthomonad cultures
tions with hydrolysed zones
reaction around or under the c
hydrolysis cannot be used as a
cultures there was sufficient var
of starch hydrolysis as a specifi
of "soluble" starch the zones o
potato starch. Some of the
reported by other workers, e
(1943), *X. hyacinthi* and *X. tr*
tive hydrolysis and *X. manihot*
hydrolyse starch. The fact that
of different workers using esse
the unsuitability of starch hydr
monas.

Because Burkholder and Li (
difference between isolates of
X. vesicatoria var. *raphani* hy
not, a special test was made w
starch media. The results wer
2 weakly positive (i.e., hydroly
beyond colony edge), 22 pos
(b) Pepper isolates (15), 2 n
zones of hydrolysis. (c) *X. ve*
hydrolysis. While there was n
strong hydrolysers of starch the
between the isolates from the d

Hydrolysis of Aesculin

Complete hydrolysis was judg
under U.V. light. The variation
only between species, but withi
cultures completely hydrolysed
30 days. Some non-xanthomon
30 days. However because of th
delayed until after active grow
value for screening xanthomona
test has no value for differentia

Hydrolysis of Sodium Hippur

Twenty-five cultures including
their ability to hydrolyse sodiu
presence of benzoic acid crysta
testing after 5 and 10 days' incu

Lipolytic Activity

37/57 cultures tested were a
Since many species were represen

this was confirmed in these tests since
ur reaction in 2–4 days equivalent to that
ı dihydrogen phosphate. No species differ-
:est.

N

broth media if shaken during incubation
.n agar slopes of similar media. Unshaken
ın slowly presumably due to slow growth
gen sulphide remaining in solution. Pre-
though lead acetate strips were more sensi-
for indicating the presence of H_2S, strips
in 3–6 days sometimes exhibited in 6–14
ɜulphide to a white or colourless substance,
:arbonate and therefore appeared negative
·easons that cultures were grown in broth
and recorded after 3, 6, and 14 days.

l cysteine with the production of H_2S and
ıroduced H_2S from peptone; most cultures
e the exceptions being 1/3 *X. begoniae,*
arum, 2/2 *X. punicae,* 1/1 *X. trichodesmae,*
icola. These results do not agree completely
shown in Table 4. This merely serves to
t cannot be used for the differentiation of

..Production Determined by Various Workers

.esult	Reference	Result Here*
ıtive	Dowson (1957)	Positive 1, 2, & 3
.tive	Ark (1946)	Positive 1, 2, & 3
A ʒative B ght	Bergey (1957)	Positive 1, 2, & 3
ıtive	Dowson (1957)	Positive 1 & 3 Variable 2
le	Bergey (1957)	Positive 1, 2, & 3

ı

Hydrolysis of Starch

The xanthomonad cultures gave results which varied from strong reactions with hydrolysed zones extending 14 mm from the colonies to no reaction around or under the colonies. Therefore it is considered that starch hydrolysis cannot be used as a generic character of *Xanthomonas.* Between cultures there was sufficient variation in reaction to suggest the unsuitability of starch hydrolysis as a specific character within the genus. In the presence of "soluble" starch the zones of hydrolysis were invariably wider than with potato starch. Some of the results were completely opposite to those reported by other workers, e.g., *X. taraxaci* reported by Niederhauser (1943), *X. hyacinthi* and *X. translucens* by Dowson (1957) as giving positive hydrolysis and *X. manihotis* reported by Elliott (1951) as unable to hydrolyse starch. The fact that marked variations occur between the results of different workers using essentially the same test is further evidence of the unsuitability of starch hydrolysis as a specific character within *Xanthomonas.*

Because Burkholder and Li (1941) reported "a clear cut and distinctive" difference between isolates of *X. vesicatoria* in that tomato isolates and *X. vesicatoria* var. *raphani* hydrolysed starch whereas pepper isolates did not, a special test was made with 44 isolates of this species in both the starch media. The results were (a) Tomato isolates (28), 4 negative, 2 weakly positive (i.e., hydrolysis under colonies but not more than 1 mm beyond colony edge), 22 positive with zones varying from 2–14 mm. (b) Pepper isolates (15), 2 negative, 10 weakly positive, 3 with 2 mm zones of hydrolysis. (c) *X. vesicatoria* var. *raphani* (1) strongly positive hydrolysis. While there was no doubt that the pepper isolates were not strong hydrolysers of starch there was in these tests no clear cut distinction between the isolates from the different hosts.

Hydrolysis of Aesculin

Complete hydrolysis was judged by the inability of cultures to fluoresce under U.V. light. The variation in rate of hydrolysis was considerable, not only between species, but within species. Approximately two-thirds of the cultures completely hydrolysed the aesculin within 18 days and all within 30 days. Some non-xanthomonads were unable to hydrolyse aesculin in 30 days. However because of the uncertainty of the meaning of hydrolysis delayed until after active growth has stopped the test would have little value for screening xanthomonads from non-xanthomonads. Certainly this test has no value for differentiating *Xanthomonas* species.

Hydrolysis of Sodium Hippurate

Twenty-five cultures including some non-xanthomonads, were tested for their ability to hydrolyse sodium hippurate. Since no culture showed the presence of benzoic acid crystals in either medium by either method of testing after 5 and 10 days' incubation, no further tests were carried out.

Lipolytic Activity

37/57 cultures tested were actively lipolytic while 8 proved variable. Since many species were represented by only 1–4 cultures, the number in the

variable category would probably increase if more cultures of these species were tested. Due to variation it is considered that this test at least when using fat emulsion agar has little value in the classification of *Xanthomonas*.

TYROSINASE ACTIVITY

The following cultures produced the dark brown pigment (presumed to be malanin) which was regarded as evidence of tyrosinase activity; Q21 (*X. campestris*), YB1, YB2 (*X. geranii*), YG1, YG2 (*X. punicae*), YR1 (*X. cajani*), ZJ1–ZJ9 (*X. pelargonii*), ZK4–ZK7 (*X. phaseoli* var. *fuscans*), ZM2, ZM3 (*X. ricinicola*). This activity is easily detected but has little value for diagnostic purposes.

ACTION ON URIC ACID

Twenty *Xanthomonas* cultures (5 species) and 5 non-xanthomonads were tested. Clear zones formed around all colonies within 43 h. After 3 days plates were flooded with 2N HCl and the zones remained clear. If the zones had been due to alkali having dissolved the uric acid, they would have become cloudy. Since this did not happen, clearing was probably due to the formation of urea. There was no precipitation of ammonium urate by any culture. Since all cultures gave a similar result this test was not considered to be of value for the differentiation of xanthomonads and no further testing was done.

ACTION ON NUCLEIC ACID

Hydrochloric acid precipitated free nucleic acid so that the medium became white and opaque except where exoenzymes caused clear zones around colonies. Most cultures hydrolysed the nucleic acid especially if the incubation was carried out over 5 days. There was considerable variation in the strength of positive reactions given by cultures of the same species. It was therefore considered that the action on nucleic acid is unlikely to be a useful differential character of xanthomonads.

ACTION ON EGG YOLK

The production of opacity in serum and in egg yolk has been shown in several instances to be caused by a lecithinase which liberates phosphorylcholine from lecithin. (MacFarlane and Knight, 1941; MacFarlane, 1948; Chu, 1949). It has also been shown that such opacity production is not limited to organisms that can elaborate lecithinase but can be attributed to lipolytic activity on certain triglycerides (Oakley, Warrack and Clarke, 1947; Gillespie and Alder, 1952). Also, Kushner (1957) found in a concentrated enzyme preparation from *Bacillus cereus* a heat stable substance that produced turbidity without having lecithinase activity. As no reference could be found regarding the action on egg yolk by xanthomonads, a short study was made in which xanthomonads, non-xanthomonad Gram-negative organisms and *Bacillus cereus* were included. The types of reaction varied so considerably within and between the 3 groups that the action on

egg yolk could not differentia
from non-xanthomonads. Sin
Liu (1961) have reported t
Xanthomonas (3 out of 4 tes
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There is no doubt that the p
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from cysteine. Asparagine is n
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egg yolk could not differentiate the *Xanthomonas* species or xanthomonads from non-xanthomonads. Since this work was carried out, Esselman and Liu (1961) have reported that many Gram-negative bacteria, including *Xanthomonas* (3 out of 4 tested) are active producers of lecithinase. This report further supports the suggestion that the action on egg yolk is not likely to be useful in the identification of xanthomonads.

DISCUSSION

There is no doubt that the phytopathogenic xanthomonads form a remarkably uniform group as shown by their cultural, physiological, and biochemical characters. The cells are non-sporing, Gram-negative, strictly aerobic, monotrichous rods. A yellow growth (with one exception—*X. manihotis*) is produced on agar and a copious slime is formed if the agar contains glucose. The metabolism of glucose is strictly oxidative. In a weakly buffered medium acid is produced in small amounts from many carbohydrates but not from rhamnose, inulin, adonitol, sorbitol, dulcitol, inositol, or salicin. Acetate, citrate, malate, propionate, and succinate are utilised but not benzoate, oxalate or tartrate. Gluconate may be utilised after a delay. No species produces an acid reaction in milk nor is there any formation of acetoin, indole, or urease. Hydrogen sulphide is formed from cysteine. Asparagine is not utilised as the sole source of carbon and nitrogen, and growth does not occur in the presence of 6% sodium chloride or at 40°c in YS broth. In all other tests included in this study there was either sufficient variation within species, or insufficient difference between the reactions of xanthomonads and non-xanthomonads, that their use for the definition of *Xanthomonas* or of its species was considered unreliable. Where the characters were found to be variable there was in many instances as much variation among cultures isolated from the same host as between those causing disease on the various hosts. Differentiation of the species which have been proposed is not possible using any or all of the foregoing tests. The key to the species of *Xanthomonas* given in Bergey's Manual (1957) becomes untenable if, as claimed herein, no xanthomonads accumulate nitrite from nitrate, most produce ammonia from peptone, all liquefy gelatin (with one special exception—*X. axonopodis*) and starch hydrolysis is unreliable.

There have been attempts to clarify the genus by serological methods and although this work has not been extensive it also shows a close relationship between species (St John-Brooks, Nain and Rhodes, 1925; Sharp, 1927; Link and Link, 1928; Link, Edgecombe and Godkin, 1929; Elrod and Braun, 1947 a, b, and c). In the last paper (1947c) Elrod and Braun reported that the antigenic properties appear to vary independently of the ability to infect given host species. Also it has been suggested that bacteriophage typing could be used for identifying xanthomonads and work to this end has been reported by Katznelson and Sutton (1951, 1953), Sutton and Katznelson (1953), Katznelson, Sutton and Bayley (1954), Eisenstark and Bernstein (1955), Sutton, Katznelson and Quadling (1958). At present

there is insufficient evidence to establish a taxonomy incorporating the serological or bacteriophage characteristics of xanthomonads though both are potentially important aids in diagnosis and classification. Colwell and Liston (1961a) recommended the use of electronic computers to aid the classification of bacteria. They noted that the 15 *Xanthomonas* species tested could be divided into two related groups and that in one group 5 species are so closely related that they form one taxonomic species. It would appear that electronic computers also will be unable to differentiate the present *Xanthomonas* species. Colwell and Liston (1961b) conclude that "there is a great deal of synonymy in this genus."

The diagnosis of xanthomonad species therefore depends solely on the host specificity claimed for the various organisms and since the host ranges have never been completely determined, the validity of the classification remains to be established. There is some support for the opinon that xanthomonads may not be strictly host specific (White, 1930; Dye, 1958; Sabet, 1959; Starr, 1959; Klement and Lovrekovich, 1961; Starr and Dye, 1963) suggestive of an unstable virulence which is regarded as an inadequate basis for the differentiation of species in other groups. The practice of forming a species solely on the basis of the host of original isolation has commonly been found to be unsatisfactory. It fails to allow the identification of avirulent strains or the easy recognition of organisms isolated from various sources or of unknown origin. For these reasons it is considered that the identity of species within the genus *Xanthomonas* is open to question. It may be suggested that the many species generally listed at present (Stapp, 1956; Bergey, 1957) could well be regarded as a single species comprising special forms adapted to particular hosts. Further investigation is necessary to determine the most susceptible hosts, the host range, and the mechanisms of pathogenicity of xanthomonads. These, together with bacteriophage and serological techniques may be required for the further clarification of species in the genus *Xanthomonas*.

Other species not included in this study should be similarly tested and such cultures would be welcomed by the writer.

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A CHEMICAL SURVEY
DISCHARGED FR
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BY W. A. J. MAHON, Dominic
Industrial

(Received for

The results are given for a che from drillholes and selected hot spr of the hot water at Kawerau is si Waiotapu. Selected chemical resul Waiotapu illustrate this point. The hole discharges at Kawerau are app in steam from Wairakei drillholes. of hot fluids at depth in the Kawer and enthalpy figures for the drillh that the main hydrothermal system present bore field.

IN

Scientific investigations at Wai generation scheme in that area, parison of the chemistry of h springs in different thermal area thermal areas so far investigated Kawerau) produce a steam/wate type of system in Italy where dri

A full description of the i Kawerau was given by Studt (1 the geophysics of the area, also the Onepu hot springs and some tion drilling by D.S.I.R. and Min

Chemical surveys of the steam drillholes at Kawerau were carri by chemists and technicians fron the initial chemical surveys, when (Mahon 1961) were not availabl times found to be contaminated anions or cations in the contamina middle of last year the Tasman installed appropriate sampling p